

Higher education crisis (cont'd)

Contradictions built into Britain's system of higher education seem to have persuaded the government towards structural change. One ingredient should be freedom — even the freedom for institutions to become extinct.

It is not every day that national higher education systems are augmented by the addition of 25 autonomous institutions, but that is what happened in Britain on 1 April. The provisions in last year's Education Reform Act that replaced the University Grants Committee (UGC) by the untried body called the Universities Funding Council also transferred responsibility for Britain's 25 polytechnics from local education authorities to 25 free-standing boards of governors, whose public support will be channelled through a similar funding council. On the face of things, Britain has for the first time a system of higher education with institutions whose diversity compares with that elsewhere.

This development inevitably marks the beginning of yet another upheaval in British higher education, but one whose consequences cannot be foreseen. But on the face of things, the two sectors of higher education will be differently affected. The universities fear that the new regime will bring even more direct control from the centre: the act gives the government powers to issue general directions to the funding council, which itself has powers to assure itself that the funds which it disburses are spent in ways of which it approves. By contrast, the polytechnics generally welcome the new regime. At the least, they will be rid of the bumbledom of local authority control. Many may even be able to work out for themselves distinctive, even novel, ways of contributing to the higher and professional education of a population increasingly

short of skill. But that is merely how it seems at present. For while the provisions of last year's act are entering into force, the British government seems already to be flirting with even more radical proposals for change.

The self-contradictory decisions of successive governments going back for exactly a quarter of a century (to the then government's enthusiastic endorsement of the Robbins recommendations that the numbers of students in higher education should be increased by a factor of 2.5) have left a hopeless muddle. The decision that the Robbins expansion required the creation of new universities has left Britain with too many universities that are too small justly to claim the title — and which are now being further robbed of academic independence by UGC's otherwise sensible attempts at rationalization.

The decision, just a few years later, that 26 polytechnics (one has since dropped out) should acquire equal status, but be managed differently, may have been most notable as a sign of emerging political animus against the universities, but it created a legacy of division (known as the 'binary system') which even last year's Reform Act left untouched. The same quarter of a century has sanctified the notions that public subventions of universities cover the costs of maintaining a capacity for carrying out research, that eligible students are entitled to maintenance grants (which are nevertheless inadequate for their stated purpose), that tuition fees for home students (now including the EEC) should be way below the economic cost and that academics should be systematically underpaid. Most governments faced with these problems have behaved as if they would prefer to start from somewhere else. The present government, with its reputation for radicalism to preserve, seems now to have woken to the need to cut the knot. After years in which it appeared to be concerned only with the money costs, it now allows that the origins of its difficulties may be structural.

So they are. There is, for example, a glaring contradiction in the university sector between the need that resources should be used effectively and the need that universities should shape the patterns of their own activities. In the first case, UGC prudently, in its last few years, took steps to see that the teaching in fields such as Russian language, agriculture and philosophy should be concentrated at particular universities, chosen (controversially) for their scholarship. A similar reorganization is now under way in the Earth sciences, and there are now pro-

Computer subscriptions

Even marvellous machines have bugs.

FROM this issue, new arrangements have been made for maintaining and fulfilling subscriptions to *Nature*. Subscriptions in the United States and Canada will be handled by JCI Data Processing Inc. of Delran, New Jersey. For the rest of the world excluding Japan, the maintenance and fulfilment of subscriptions have now been transferred to *Nature's* own computer system equipped with customized VISTA software. While there is every confidence that the end result will be a better service for subscribers, their indulgence for the customary unforeseen problems is earnestly requested. Arrangements for Japan continue unchanged. □



posals that rationalization (as it is called) should be extended to the teaching of physics, chemistry and biology (see *Nature* 338, 363; 30 March 1989). But two foreseen difficulties are now apparent. The cost of reorganization is itself high. And the end-point of the process must be that few British universities will be able to offer a range of studies wide enough to justify their social and academic functions. Is that not a high price to pay for a fudged remedy for mistakes made a quarter of a century ago?

The polytechnics, new-found delight with titular independence notwithstanding, may soon find themselves in a similar case. While their bread-and-butter teaching at the undergraduate level will continue to be supported by their funding council, many of them appear to believe that their future lies in the further development of professional and continuing education, usually of a technical or technological character, for which there is a crying need. More directly than the universities, they will be exploring the now non-existent British market in adult education. The clever among them will no doubt be able to forge profitable links with the network of new technical training councils which the government has separately proposed. But they, too, may then find that the end result is that they have exchanged one kind of thralldom (to local authorities) for another.

The support of research in this changing pattern raises other contradictions. The British research councils make project grants to polytechnics as well as to universities, although the bulk of what they choose to spend in this way ends up in universities. (Part of the explanation is that polytechnic budgets are not reckoned to cover the cost of maintaining research capability, notionally 30 per cent of UGC's recent subventions, which is why they seemed to the Labour government of 1966 to be more "cost-effective".) But since the early 1970s, all the research councils have been spending too great a proportion of their resources on central facilities and too little on research grants, while universities have more recently taken to squirrelling away research money to keep body and soul together. The process has now gone so far that the Science and Engineering Research Council, the largest of the four concerned with science, seems to have put its responsibilities towards individual researchers second after its enthusiasm for directed research (see *Nature* 337, 291; 27 January 1989). So why bother to maintain a capacity for research at universities?

If the British government seriously intends to resolve these issues, it needs some guiding principles. Here is a short list to begin with.

■ **Autonomy.** The British government and its new funding councils must recognize that academic institutions are themselves the most reliable judges of how all kinds of higher education should develop. (Civil servants have a poor track record in this role.) But good judgement requires both the freedom and the size to respond flexibly to changing circumstances. The right to decide what to teach, and how, the cornerstone of academic freedom, is the best guarantor of continuing good sense. Institutions'

dependence on public funds is neither here nor there: the University of California is not noticeably compromised by decisions made by the government of California, which foots the bill. The principle applies as much to the polytechnics as to the universities.

■ **Incentives.** Means must be found of rewarding successful institutions. Much of the present mess in British higher education stems from the way in which institutions have been deprived of a sense of opportunity for close on 20 years. The regulation of capital funds and even of student numbers have been used to constrain the sizes of institutions (keeping too many of them too small). The government should now be prepared to see this relationship turned inside-out. While growth is not everything in academic life, institutions wishing to grow and able to attract the students should be enabled to do so. The other side of that coin is that unsuccessful institutions may shrink, even to the threshold of viability, which may be politically uncomfortable. But how else can a government which believes that market forces discover truth conduct itself?

■ **Research.** Reputation in research is another legitimate goal for academic institutions (and a powerful means by which the quality of its teachers may be generally improved). Research is also, of course, valuable in its own right. But this reward has also, in the past decade, been denied by the scarcity of funds, likely to be further restricted by the research councils' ever-greater fondness for directed research. And in the university sector, there is a prospect that the implicit distribution of research overhead will now be entirely out of pattern with the planned concentration of research. This is why it will seem attractive to the government to arrange for a wholesale transfer of funds of research support in universities from the funding council to the research councils, but that would be a mistake. The more urgent need is to come to a clear decision about the responsibilities of the research councils towards research in both the universities and polytechnics, and then to let institutions of all kinds find their appropriate levels. Less successful institutions may find themselves losing in the competition for survival, but to deny them the opportunity of reversing that trend would be destructive.

■ **Money.** On past form, the British government would probably believe that it had discharged its responsibilities to higher education if it could persuade other people to meet the costs, but the inference is incorrect and the goal absurd. Exhortation will not have British industry or parents supporting higher education the day after tomorrow; even tax incentives on the US pattern would probably have only a small effect (which is no reason for not trying them). But there is a case for changing radically the ways in which funds are at present channelled to institutions. Higher tuition fees (paid for indirectly by central government) are the obvious means by which universities could compete for students and so win back their freedom — even their freedom to go out of business. □

Cold fusion causes frenzy but lacks confirmation

- Worldwide attempts to reproduce results
- \$5 million may be given for US research

Washington, Tokyo & London

REMARKABLE reports of "nuclear fusion in a test-tube" achieved by two laboratories in Utah have prompted a frenzy of activity in laboratories around the world as others try to replicate the experiment.

First news of the reported breakthrough in 'cold fusion' came from a press conference on Thursday, 23 March, at the University of Utah in Salt Lake City. Stanley Pons, a professor of chemistry at Utah, and his colleague Martin Fleischmann from the University of Southampton reported that they had achieved fusion by passing a current through a palladium electrode in an electrolyte solution in deuterated water. By the next day, on the basis only of newspaper accounts of the experiment, laboratories around the world were scrambling to copy the experimental design.

At the same time, traffic started flying across computer networks as interested chemists and physicists sought details of the experiment. By the end of last week, preprints of papers prepared for publication by both Pons and a second Utah group, led by Steven E. Jones of Brigham Young University in Provo, were available on electronic bulletin boards.

Numerous laboratories in the United States have attempted to replicate the findings. Los Alamos National Laboratory, Lawrence Livermore National Laboratory, IBM in Yorktown Heights, New York, AT&T Bell Laboratories in New Jersey and the University of Illinois at Champaign-Urbana are just some of the institutions that are said to be looking into the Utah claims, although none has reported unequivocal confirmation. The price of palladium has reached new heights on commodity markets.

Professor Noboru Koyama of the Tokyo University of Agriculture and Chemistry reported at a meeting of the Chemical Society of Japan on 1 April that his team had detected very large amounts of heat and gamma rays in an experiment two days earlier when they passed an electric current between palladium and platinum electrodes in an insulated tube filled with heavy water. However, Japanese experts who have seen Koyama's results expressed scepticism about the validity of the claim because no measurements of neutron yield were made. Koyama says he will try to duplicate the experiment with the Japan Atomic Energy Research Institute to determine neutron yield.

In Britain, teams from the University of

Birmingham and the Rutherford Appleton Laboratory and the UK Atomic Energy Authority at Harwell are attempting to confirm the results, and two scientists at the University of Debrecen in Hungary, Gyula Csikai and Tibor Staricskoi, claim to have obtained the same results as Fleischmann and Pons, but no details of their experiment are yet available.

In Italy, the Ettore Majorana Centre for Scientific Culture has announced a

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Pons and Fleischmann: handling it carefully. world conference for next week (12 April) in Erice to discuss the cold-fusion experiments. Europe, the Soviet Union and the United States are about to launch a ten-year, \$1,000-million project to study the viability of fusion by inertial confinement using X-ray lasers, but all may change pending the conclusions of the conference.

Press interest, as well as interest from the scientific community, remains at a fever pitch. At a seminar last week at the Salt Lake City campus, Pons presented additional details of the experiments to a packed lecture hall. He reported that his laboratory had achieved even greater energy efficiency from the experimental apparatus, although he joked that it would be 100 years before the technology could be made commercially available. Pons warned those trying to replicate the experiment to be extremely careful, as his own early attempts had led to heat generation that had run out of control.

The Brigham Young group has been less forthcoming in public about their results. They believe they are seeing a signal indicating that the deuterium atoms are fusing inside the palladium lattice, although they have not seen the same energy output recorded by Pons and his colleagues.

The US Department of Energy (DoE) has been interested in research in cold fusion for some time. According to a DoE

spokesman, the agency has spent some \$9 million in this field, with \$1.9 million going to Jones. A proposal from Pons submitted last fall for DoE support for a project to "further explore nuclear fusion reactions in deuterium-charged palladium" was approved by DoE's Advanced Energy Projects Division, and \$322,000 is expected to be released for the project later this spring.

The state of Utah has shown even greater enthusiasm. The day after the University of Utah press conference, Governor Norm Bangerter announced he would convene a special session of the state legislature for the sole purpose of considering a supplementary budget request of \$5 million for a new institute to pursue research in cold fusion. National Aeronautics and Space Administrator James Fletcher has agreed to sit on an advisory panel for the new institute once it is created. A spokesman for Bangerter says the new money is contingent upon confirmation of the Pons experiment, but it is unclear whether that will come before the legislature holds its session tomorrow (7 April). □

AUSTRALIA

Human embryo experiment banned

Sydney

THE state government in Victoria has overturned a decision to permit a controversial experiment on human embryos and one member of the committee to regulate *in vitro* fertilization (IVF) has resigned in protest. The experiment, designed to test human embryos for birth defects before implanting them in patients, would have been the first to involve embryos older than 22 hours. Dr John Henley, who resigned, is a theologian and master of one of Melbourne University's colleges. He has been a member of the Standing Review and Advisory Committee on Fertility, chaired by Monash University's Professor Louis Waller, from its inception.

The experiment was to have been carried out by the IVF research group at Monash University, headed by Dr Alan Trounson. It would have involved the biopsy of 11 embryos that had been slow to grow. Trounson says that the experiment was approved unanimously by the committee. "It even passed without comment from Father Harmon (the representative of the Roman Catholic Church), who is not allowed to approve of anything."

According to Trounson, the government's intervention came in response to public pressure, provoked by newspaper reports saying that the experiment would lead to genetic engineering and cloning. He says that "the government's action is an unfortunate politicization of the whole exercise".

Charles Morgan

AP Laser Photo.

Release of sea-floor maps

Washington

THE US Navy made a U-turn last week and announced that it will permit the release of most of the detailed sea-floor maps of the US Exclusive Economic Zone (EEZ) prepared by the National Oceanic and Atmospheric Administration (NOAA). The announcement follows rumours that the Navy was steaming in the other direction and was about to condemn all high-resolution bathymetric data collected in federal programmes to the same fate as the NOAA data, classified by a White House memo from Vice Admiral John Poindexter in 1985.

The change in policy comes as a welcome relief to oceanographers who were living "under threat that our data might become classified and that we would not be able to freely distribute our results" according to Ken Macdonald of the Marine Science Institute at the University of California at Santa Barbara. The NOAA maps are likely to be an exciting data source, he said, providing detailed information on many scientifically interesting areas off the west coast.

Restrictions on the NOAA maps will not be lifted for all of the 130,000 square kilometres of sea bed they cover. Excluded will be areas, of yet undefined size, around ballistic missile submarine's home ports. The Navy's own massive collection of high-resolution bathymetric data will definitely remain classified. Most of it is believed to be for the deep oceans (the location of surveyed areas is also classi-

fied) and would be of enormous value to oceanographers. But the Navy argues that it needs the data kept secret as it uses it to determine the best sites for sensors to detect enemy submarines and to map the gravity anomalies which affect the flight of ballistic missiles. Occasional glimpses of part of the Navy data have been given on a "need to know" basis, producing, for example, the map of the North Atlantic sea mounts published by David Epp and Christian Smoot (see *Nature* 337, 254; 1989). But even in this case the location of the sea mounts was not given with full accuracy.

The Navy's tough policy on bathymetric data has done little good to US industry. General Instrument Corporation, the major US maker of multibeam sonar, has had to refuse several export orders, while Finnish, German and Norwegian companies have sailed away with business. The damage to US industry, plus the knowledge that it was becoming possible to buy foreign sonars that would map the sea floor as accurately and faster than NOAA's equipment, may have helped to prod the Navy into a more open attitude.

Most oceanographers attribute the change in policy to last year's appointment of Rear Admiral Richard Pittenger as Oceanographer of the Navy. Despite his background in anti-submarine warfare, he is believed to value academic research. But the Navy keeps quiet about its policy decisions and will not give reasons for its change in course.

Alun Anderson

RADIOTELESCOPE

Green Bank failure identified

Washington

THE cause of the sudden collapse of the 300-foot radiotelescope at Green Bank, West Virginia, last November (see *Nature* 296, 336; 1988), on a night without wind, rain or snow, has been traced to the fracture of a single, highly stressed steel connection plate, according to an independent inquiry which submitted its findings to the National Science Foundation last week.

That conclusion leaves the National Radio Astronomy Observatory free to clear away the enormous tangle of steel that had been left undisturbed while the inquiry was in progress. But it makes it no easier to sort out the scientific and political issues surrounding the question of whether the telescope should be rebuilt.

The three-man inquiry panel found no evidence of inadequate maintenance of the connection plate, or of improper operation of the telescope. But modern computer structural stress analyses, show that stresses in many parts of the

telescope "were substantially higher than is permissible today".

The telescope's design problems can be overcome using modern techniques, says the report, and provide no technical obstacles to its replacement. West Virginia's two Democrat Senators, Robert C. Byrd and John D. Rockefeller, are already demanding that a similar, state-of-the-art telescope be built as the "best promise for jobs, education, tourism and scientific prestige" in a state with little else to offer in the way of large, basic research facilities. Byrd, who holds a powerful position in the Senate Appropriations Committee, says he "will aggressively pursue funding" for the telescope. Plans for a supplemental appropriation to the NSF budget for a new telescope are already under way.

But astronomers are not necessarily sure that replacing Green Bank would be the best use of the \$75 million required. The large telescope was valuable for survey and mapping and was able to detect

Protest as Pasteur speaks English

Paris

A DECISION to give the Pasteur Institute's journal an English title has caused confusion and protest in the French press, but seems to have left the scientific community unscathed. Under the banner "Goodbye Pasteur!", the French national newspaper, *Le Monde*, broke the news that, in future, the *Annales de l'Institut Pasteur* will be published only in English, for the first time since it was created in 1887. This, ran the article, sounds the death-knell for French-language science.

Two changes have been made to the journal. The title will now be *Research in ...* (immunology, virology or microbiology, according to the discipline covered). Second, the review panel has been widened to include non-Pastorians and foreign scientists. But, says the Pasteur Institute, articles submitted in French and accepted for publication will be printed in French, while articles in English will continue to have a French abstract. For the press, the changes signal the disappearance of a flagship of French science under the 'tyranny' of the English language. But the Pasteur Institute sees things differently. "In 1973 only 10 to 15 per cent of the articles were received were in English, but in 1987 the proportion was almost 100 per cent", says a spokeswoman. "But of the articles we published, 58 per cent were from French-speaking authors and over 80 per cent of these were originally written in English. The title of the review, however, suggested that it was not open to the scientific community as a whole and so researchers started to send their papers elsewhere. The *Annales de l'Institut Pasteur* has to survive."

Peter Coles

faint sources, including a pulsar in the Crab nebula. But according to Kurt Riegel of NSF's Division of Astronomy, a decision to rebuild the telescope would face competition from projects to upgrade the radiotelescope at Arecibo, to build large optical telescopes and to enhance the image-processing capabilities of the National Radio Astronomy Observatory's very large arrays.

If a new radiotelescope is not built, "it does not bode well for the site at Green Bank", according to the facility's director, Paul A. Vanden Bout. A 140-foot radiotelescope remains at Green Bank but it is 26 years old. A possible solution, already suggested by NSF officials, is to build the eastern end of the proposed Laser Interferometer Gravitational Wave Observatory (LIGO) at Green Bank. The project requires a facility in California and in the east to be run in coincidence. But Byrd and Rockefeller say that while they welcome LIGO, they want the radiotelescope replaced too.

Alun Anderson

CANCER THERAPY

Cancer data are puzzling

Washington

THE US General Accounting Office (GAO) has uncovered a conundrum in breast-cancer therapy. Although there is good evidence from randomized clinical trials that chemotherapy following surgery is beneficial for premenopausal women with breast cancer that has spread to the lymph nodes, increased use of chemotherapy has not been followed by decreased mortality statistics. In 1987, Congress asked GAO — its investigative agency — to determine whether cancer patients were actually receiving the latest therapies being developed with federal money by the National Cancer Institute. In its latest report*, GAO used data from the Surveillance, Epidemiology and End Results (SEER) programme to track mortality from breast cancer from 1975, when chemotherapy was first shown to be an effective treatment, until 1983, the latest year for which complete data are available.

Although there is still some debate over the value of chemotherapy for breast-cancer patients whose cancer has not spread to the lymph nodes, clinical trials have shown that for node-positive premenopausal women, chemotherapy is beneficial. Nevertheless, despite an increase in the number of women receiving chemotherapy between 1975 and 1983, there is no detectable change in survival figures. The analysis confirmed that age and race are significantly related to the probability of dying from breast cancer.

GAO proposes three explanations for its results. The first, and least likely, is that chemotherapy is not so beneficial despite studies to the contrary. A second possibility is that doctors are not giving appropriate or sufficient drugs to their patients, and a third possibility is that the improvement that can be expected from chemotherapy is not great enough to be detected by GAO's statistical techniques.

But Vincent DeVita, Physician-in-Chief at Memorial Sloan-Kettering Cancer Center and former director of the National Cancer Institute, says GAO is premature in raising the long-term survival issue. He argues that only a small fraction of the appropriate patient population was receiving chemotherapy in 1975, and newer therapies will yield a more encouraging picture in a few years. DeVita accuses GAO of designing its study to validate preconceived ideas.

The GAO report calls on the Department of Health and Human Services to conduct a larger study to explain the curious findings.

Joseph Palca

* Breast cancer: patients' survival. United States General Accounting Office, GAO/PEMD-89-9, Washington, DC, 1989.

RESEARCH ANIMALS

Activists infiltrate Stanford

Berkeley

THE Stanford University biology faculty has become involved in a new controversy over the use of animals in research by criticizing an undergraduate course taught by two animal rights activists. A letter from the biology faculty objecting to the course was obtained by the two instructors, who are now demanding an apology.

Stanford has had difficult relations with animal rights activists for the past several years. Protesters have caused more than a year of costly delays for Stanford in building both a new animal facility and a new biology research building (see *Nature* 329, 477; 1987). So the biology faculty was dismayed to learn last autumn of a Stanford course called "The Case for Animal Rights", being taught by Kimberly Sturla



and Lise Giraud of the Peninsula Humane Society, both of whom have been involved in the building protests.

The course was part of a popular programme called Stanford Workshops on Political and Social Issues (SWOPSI), intended to expose students to controversial subjects. Biology chairman Philip Hanawalt says department members did not object to a course on animal rights, but were concerned because one of them who had attended the class reported that the instructors showed ignorance of the scientific arguments for using animals, and created a hostile environment for speakers invited to present that view. The faculty voted unanimously that a letter of objection should be sent to the dean of undergraduate studies.

"We decided that it is the prerogative and duty of the academic faculty to maintain quality control in what is taught to Stanford students", said Hanawalt, "and therefore it was entirely appropriate for us to make comments on this." Those who observed the class and wrote the letter have requested anonymity out of fear of reprisals by the activists.

The letter, which was addressed to

Thomas Wasow, dean of undergraduate studies, with a copy to Margo Horn, director of SWOPSI, questioned the qualifications and scientific knowledge of the instructors, and accused them of requiring ideological compliance of the students and of failing to present both sides of the issue. When the instructors heard of the letter and asked for a copy, Horn consulted Wasow, who agreed that she could send them one. Wasow says that at the time he did not consider the letter, which bore the names of the entire biology faculty, to be confidential. Hanawalt says such internal memoranda should be considered confidential. He compares its release to the instructors to the release to a faculty member denied tenure the letters upon which that decision was based.

Through their attorney, Sturla and Giraud have publicized the letter, called it "defamatory" and demanded an apology. Sturla claims she is "very qualified", to teach the course, and points out that the course dealt with issues other than research, such as the use of animals for food and in rodeos. She adds that in each case, experts were brought in to present each side of the argument.

David Maurice, a professor of ophthalmology who spoke in favour of animal use in research, says he was given a polite hearing, but felt that his words fell on deaf ears, as the students had already been strongly biased against animal use. Maurice, who himself co-taught a seminar on alternatives to animal use several years ago, questions whether activists like Sturla and Giraud are the right people to teach such a class.

Marcia Barinaga

SCIENCE POLICY

Brazilian appointment

São Paulo

CONTINUING its quick-change approach to science and technology policy, the Brazilian government last week selected a secretary for its new Special Secretariat for Science and Technology, a cabinet-level body created just two months after the sudden extinction of the Ministry for Science and Technology. The appointment of Décio Leal de Zagottis, director of the influential Polytechnic School of the University of São Paulo, may signal a change in computer-industry policy. In the two months following the death of the old ministry, science policy and the management of several government institutes went to the Industry and Trade Ministry, whose minister, Roberto Cardoso Alves, opposes Brazil's protectionist stance towards the computer industry. The new secretary says he will oppose change.

Ricardo Bonalume Neto

Proposal divides biologists

London

BRITAIN'S biology community is divided on the merits of the new plan to restructure biology teaching and research in universities (see *Nature* 338,363; 30 March 1989). Some are resigned to the plan, others will strive to persuade the Universities Funding Council (UFC) to modify some of the proposals.

The report, distributed to universities

last week, was carried out for the University Grants Committee (which was dissolved last week) by a group led by Professor Sir Richard Southwood of the University of Oxford. It was the subject of heated discussion at the first meeting on 31 March of a new group comprising more than 50 heads of biology departments.

The most controversial aspect of the plan is the proposal to group existing

departments into two kinds of much larger departments, one focusing on molecular biology and the other on more traditional areas such as ecology and evolutionary studies. This was criticized as "disastrous", "illogical" and "an odd reversal of the current trend towards integrative biology". One speaker said it would divorce the techniques of molecular biology from the subject areas in which they were being applied. The department heads agreed that a rigid division between the two types of department would be detrimental.

On the other hand, they disagreed over interpretation of the report, some saying it was too dogmatic and others insisting that there would be flexibility.

The proposal to group all biology departments in a university into a single school of biological sciences was also welcomed. Most agreed that fragmentation is undesirable, but the report was criticized for putting forward only one model for integration. The group agreed that for different universities, different strategies would be necessary.

The group also agreed with the recommendation that pre-clinical teaching of biology in schools of medicine, dentistry and veterinary science should be the responsibility of the new schools of biological sciences. Professor James Callow, of the University of Birmingham, described this as the "most radical proposal" in the plan, but the review committee was criticized for not studying this problem in more detail. This proposal is likely to meet fierce opposition in medical schools.

The biology heads were particularly concerned about the effects on biology of the planned restructuring of other sciences. Although the biology plan recommends no closures, the expected closure of more than ten university chemistry departments is expected to have a knock-on effect on biology.

There was also concern about the future quality of entrants into university biology courses. Changes in the content of school examinations are likely to mean that students have less factual knowledge in the sciences. The biology heads also fear that the courses provided at British universities may compare badly with more comprehensive and longer courses at other European universities. One proposed solution to both these problems was that England should follow Scotland in introducing 4-year degree courses, instead of the present 3-year courses.

The committee elected by the new group of heads will put a response to the biology report formally to the new Universities Funding Council later this month. This report was intended to be only the precursor to a more wide-ranging review, but was carried out quickly so that restructuring of biology would not lag too far behind restructuring in the other sciences.

Christine McGourty

SOVIET SPACE

Phobos failure raises doubts

London

THE failure last week of Phobos-2, the second of the two Soviet Mars probes launched last July, could hardly have been worse timed for Soviet space planners. During the past few months, there has been growing public criticism in the Soviet Union of the cost of space, particularly of deep-space missions with no obvious economic spin-off. One of the chief advocates of swingeing cuts in the space budget is Mr Boris Yeltsin, who on 26 March won a resounding election victory over the 'official' candidate. The following day, contact with Phobos-2 was lost.

The two-craft Phobos mission included a wide range of investigations of Mars and its satellite Phobos, including the firing of a laser at Phobos and the landing of a 'hopping' probe to investigate the satellite's surface. It carried experiments from 13 countries and multinational agencies and had been presented as a first step to an international manned mission to Mars early in the next century. But Soviet media coverage of the mission has from the beginning been tentative.

The original mission consisted of two spacecraft, carrying different experiments. Early in September, Phobos-1 came to grief when a ground-based computer error led to its being sent an incorrect command. In December, the main transmitter on Phobos-2 failed, but the craft arrived on schedule and on 23 January was inserted into an orbit around Mars. It functioned as planned for the next nine weeks, returning several photographs of the surface of Mars and Phobos.

On 27 March, the command to rotate the probe to prepare for the close (50-m) transit of Phobos was apparently followed correctly — but mission control found it impossible to re-establish contact. Two days later, the head of the Glavkosmos space agency said his experts were working round the clock to try to restore the links. But these efforts proved fruitless.

Although Soviet newspapers have made the best of the photographs and data already received from Phobos-2, these are unlikely to restore public confidence. But

as *Pravda* revealed last week, there is equal resentment that it will be a Japanese journalist (paying his fare in hard currency) and not a Soviet citizen who will be the first media representative to visit the Mir station (see this page).

Vera Rich

JAPAN IN SPACE

Journalist plans scoop

Tokyo

THANKS to the Soviet Union, the first Japanese citizen to journey into space may be a journalist from a private television network rather than one of the astronauts training for a ride on the US space shuttle.

Last week, the Tokyo Broadcasting System (TBS) network signed an agreement with the Soviet Commission for Space Exploration (Glavkosmos) that will allow a TBS reporter to spend six days aboard the Mir space station in 1991. The reporter will make live broadcasts to Japan every day throughout his stay in the station.

The agreement comes as a shock to Japan's National Space Development Agency (NASDA), which has been planning for years to put the first Japanese astronaut in space aboard the US space shuttle. Although a final date for the reporter's space voyage has yet to be set, TBS has arranged the flight to commemorate the network's 40th anniversary on 10 May 1991. There is thus a strong possibility that TBS will 'scoop' NASDA's plans to put an astronaut on the space shuttle in July of the same year.

Soviet journalists are also upset about the agreement. *Pravda*, the Communist party daily newspaper, welcomed the Soviet authorities' decision to offer a journalist a space flight but complained that only foreigners could afford to pay the fare. The Communist Party youth newspaper *Komsomolskaya Pravda* carried, under the headline "Prestige trampled underfoot", pictures of Yuri Gagarin, the first man in space, Valentina Tereshkova, the first woman in space, and a Japanese 10,000-yen banknote with the caption "The first journalist in space?".

David Swinbanks

OIL SPILL

Shipwreck fouls the water

Berkeley

THE largest oil-tanker spill in US history has turned Alaska's Prince William Sound from a pristine environment into a disaster area. Biologists are not yet able to assess the full extent of the damage to the area's wildlife, but the political damage to those pressing for further oil development in Alaska is already plain.

On 24 March, the fully-loaded oil tanker *Exxon Valdez* struck a reef shortly after leaving the Alaskan pipeline terminal in Valdez, southern Alaska, spilling 10 million gallons of crude oil. The tanker's captain, who had been drinking before the accident, had left the ship in the hands of a crewman not certified to pilot the ship within the sound.

Little oil from the spill was recovered, and angry fishermen and environmentalists blame Exxon and the Alyeska Pipeline Service Company, which manages the pipeline, for a disorganized clean-up effort that squandered precious time. Floating booms intended to be deployed quickly to contain a spill did not reach the site until more than 10 hours after the wreck, said Jon Lyman, of the Alaska Department of Fish and Game. Alyeska officials blamed the delay on the fact that the barge intended to carry the booms was unloaded for repairs.

Alyeska spokeswoman Beverly Michaels said that skimmers and booms are not practical for such a large spill, and Alyeska was prepared on the day after the spill to apply chemicals that disperse the oil into the water. But fishermen, concerned about the toxic effects of the dispersants and dissolved oil, persuaded the Coast Guard to delay permission to use them. By the time permission was received the following day, said Michaels, high winds made it impossible to apply the dispersant from aircraft. Debate still continues over whether dispersants or containment and collection would have been best, but after two days of heavy winds the oil was spread over 550 square miles, ruling out either method. Fishermen and state officials joined Exxon and Alyeska in using barriers to protect the most environmentally sensitive areas.

Because the Valdez spill is in a relatively enclosed area, it formed a continuous blanket over the water, potentially trapping aromatic hydrocarbons, such as benzene or toluene, in the water beneath the oil. Jacqueline Michel, an environmental chemist for the National Oceanic and Atmospheric Administration (NOAA), said laboratory studies have shown such chemicals to be highly toxic to planktonic fauna.

The herring-roe fishery will be the first influenced by the spill. Within the next few weeks, the fishery would normally

begin to capture herring that have entered the sound to spawn, and allow them to deposit their eggs on kelp, which is harvested after the fish are released. Sale of this year's harvest may be banned if hydrocarbon contamination is detected in the spawning areas or in the roe that is collected.

High priority was given to protecting the economically important hatcheries where young salmon are raised and returned to the sea in May. Some are tagged each year before release, and survival data are collected when the adults are caught by fishermen several years later. Tags from those released this year will provide information on the impact of the spill on the salmon population.

Lyman said that birds and sea otters are particularly vulnerable to oil spills, because the oil destroys the insulating qualities of their fur and feathers. Exxon brought in an animal rescue team from California, but rescue attempts were hindered by the inaccessibility of the rocky beaches in the area. Tens of thousands of birds and otters live in the sound, and many of those are likely to have been harmed or killed by the oil, but a week after the spill only a handful had been rescued and cleaned. The cleaned animals will be tagged before their release, to aid biologists in determining the fate of animals following the spill.

Migratory puffins, terns, cormorants and other birds are just beginning to enter the area. Their nesting grounds, as well as the areas where sea lions will give birth in May, were included among areas to be protected with booms from the progressing oil slick.

Exxon will hire local residents to clean oil from hundreds of miles of shoreline. Exxon spokesman Brian Dunphy said absorbent pads will be used to soak up the oil, and high-pressure hoses will blast it off rocks. Michel, of NOAA, said wave action and biological activity will purge the oil from exposed sites, but sheltered areas may remain contaminated for years.

NUCLEAR SAFETY

Cuba signs agreement with Soviet Union

London

CUBA has signed a nuclear safety agreement with the Soviet Union, regulating Soviet assistance at the Cienfuegos nuclear plant and "other nuclear projects" under construction. The Soviet side is to equip Cuban nuclear safety laboratories with services, hardware and documentation, and will also arrange consultancy services on how to organize radiation monitoring. Cubans will be trained in the Soviet Union as operators of nuclear power stations.

Vera Rich

The spill is likely to cause political fallout, as the battle continues in Congress over oil development in the Arctic National Wildlife Refuge, adjacent to the Prudhoe Bay area which now supplies oil to the Alaskan pipeline. Environmental groups have opposed the development (see *Nature* 327, 9; 1987), while oil companies and the Bush administration have argued that development is essential, and can be conducted in an "environmentally sensitive" manner. The Valdez spill did not change administration policy, but environmental groups expect its effects on public opinion to be felt in Congress, where competing bills are being considered that would either open the area for development or declare it a wilderness area, thus preventing oil drilling. Opponents of oil development off the California coast expect the spill to aid their cause as well.

Although an oil spill at the pipeline terminal is not the same as the general environmental disruption feared from construction of oil exploration sites in the Alaskan wildlife refuge, Don Hellmann, of the Wilderness Society, said it raises the question of whether assurances from oil companies can be trusted. "The oil companies have been saying all along that they can drill without harming the environment, and if there is a problem such as a spill, they can take care of it right away. Now we've seen that they're wrong on both counts."

Marcia Barinaga

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Barges and tugs head to clean up the worst of the oil spill in Alaska's Prince William sound, where the *Exxon Valdez* ran aground spilling 270,000 barrels of crude oil.

Operators to cooperate

Boston

NEXT month in Moscow, the World Association of Nuclear Operators (WANO) will begin formal operations, ushering in what supporters hope will be an era of increased international cooperation among the world's nuclear power industries.

WANO was conceived initially in response to the accident at the Chernobyl reactor in 1987. Participating power plants will share information about the safe operation of their plants and the details of any "off-normal" events through a world-wide computer network. The association has already opened regional centres in Paris, Tokyo and Atlanta (Georgia), and a coordinating office in London. A fourth is due to open in Moscow later this month. In a remarkable show of solidarity, the organization will include the participation of every nuclear utility in all of the 31 countries that use the technology to generate electricity.

Through its regional centres, the group will collect data about all mishaps and untoward events at nuclear power plants, and will organize workshops and technical exchange visits between nuclear operators from different nations.

While other international organizations exist to promote the safe use of atomic energy, WANO is unique in its exclusive orientation to industry, and its goal of uniting nuclear-plant operators worldwide. WANO is modelled on an existing organization in the United States called the Institute of Nuclear Power Operations (INPO) which was also formed in response to a nuclear mishap—the partial meltdown at Three Mile Island in 1979 (see *Nature* 338,190;1989).

Greater information exchange among nuclear operators might have prevented the operator error that led to the accident at Three Mile Island, according to many INPO staff. Addressing participants at an INPO conference in Atlanta last fall, Lord Marshall, chairman of the WANO Steering Committee, stated that the accident at the Chernobyl reactor "never would have happened" if WANO had been in place and "working effectively". "Peer group pressure" would have forced changes in the "unacceptable" degree of reliance on the operator in the safety design of the Chernobyl plant, said Thomas Eckered, acting director of the London WANO coordinating centre.

Marshall stressed the importance of the choice of Moscow as the location of the inaugural meeting of the group. That, he said, shows the acceptance by the world nuclear industry of Soviet commitment "to *glasnost* and *perestroika* in their nuclear business."

Seth Shulman

Proliferation of nuclear subs

Boston & São Paulo

FEARS that the acquisition of nuclear-powered submarines by Brazil, India and Canada will blur the line laid out in the Nuclear Non-Proliferation Treaty and foster the spread of nuclear weapons to developing nations are largely unfounded, according to the majority view at a meeting held last week at the Massachusetts Institute of Technology (MIT). But military and technical experts from several nations, including those planning to acquire nuclear-powered submarines, agreed that there are real fears that deployment of nuclear submarines could trigger regional arms races in South Asia and South America.

Brazil, India and Canada are all planning to build or buy nuclear-powered submarines. India last year leased a nuclear submarine from the Soviet Union and is rumoured to be negotiating for three more, even as it continues work on its own development programme. K.K. Nayyar, retired vice-admiral of the Indian Navy, defended his nation's nuclear-submarine programme not only by emphasizing its deterrent capability but also by acknowledging India's desire for a stepped-up military presence in the Indian Ocean. "India represents 17 per cent of the population of the planet", Nayyar stated, "we want to be there to be reckoned with".

Brazil too has regional power plans. A statement from Mario Cesar Flores, an admiral in the Brazilian Navy, said that

Brazil's ambition to have a "strong presence" in the South Atlantic leads it "in a firm, prudent and deliberate fashion, to the nuclear-powered submarine". Brazil was apparently impressed by the performance of British nuclear submarines during the Falklands war.

Three conventional submarines are now being built in Brazil with West German help while indigenous efforts are made to develop small reactors and enrich nuclear fuel at the Centro Experimental Aramar at Iperó near São Paulo. According to Adrian English, a US expert on Latin America's military balance, as soon as Brazil deploys a nuclear-powered submarine, "two to four nations in the region will be sure to follow suit", including Argentina, Chile and Cuba.

The role of nuclear-powered submarines in regional conflicts seems a bigger problem than their impact on nuclear-arms programmes. Several participants at the meeting pointed out that the countries currently investigating the development of nuclear submarines already have nuclear-power programmes and "plenty" of plutonium for weapons at their disposal. Brazil's Admiral Othon Pihheiro, known locally as the "Brazilian Rick-over", claimed success for the nuclear enrichment programme last week, saying that the first batch of enriched uranium reactor fuel will be delivered at the end of the year.

Seth Shulman & Ricardo Bonalume Neto

ENVIRONMENTAL PROTECTION

Trouble at the mill in Australia

Sydney

PLANS for a \$1,000-million chemical pulp mill in Tasmania have been scrapped after the Australian government decided to impose strict environmental controls as a condition of approval for foreign investment in the project. The mill, at Wesley Vale, was to have been a joint venture between the Australian company North Broken Hill Peko and its Canadian partner Noranda.

Such environmental guidelines are usually set by the state government. It is believed that the Tasmanian government agreed to loosen its guidelines because of the employment opportunities offered by the project. But in this case, because of the level of foreign investment, the project had to be approved by the federal government's foreign review board.

At issue is the production of dioxins, which occurs when mills bleach paper with chlorine. In humans, dioxin exposure causes the skin disorder chloracne and has been linked to other illnesses. According to government sources, the companies involved in Wesley Vale were not prepared

to spend enough money to replace dioxins in the bleaching process, or to change the mill's effluent pipeline into Bass Strait.

The Minister for the Environment, Senator Graham Richardson, has come under strong pressure from the government to develop clear-cut environmental guidelines. While agreeing to do so, Richardson has stated that differing circumstances in individual cases may make them difficult to formulate.

Companies at present planning three other pulp mills in Victoria, New South Wales and West Australia, all similar in scale to Wesley Vale, are unlikely to proceed without comprehensive guidelines.

Prime Minister Bob Hawke told the Australian Broadcasting Corporation radio programme "PM" that he recognized that the loss of the mill would damage both investment in Australia and export earnings. "As long as I'm Prime Minister I won't have development at any price. Each day they would be dumping 13 tonnes of organochlorides into the ocean and I will not allow it."

Tania Ewing

Coming of age in anthropology?

Adam Kuper

With the screening in several countries of a film on the subject, the controversy over Margaret Mead's work in Samoa rumbles on. Underneath the thunder and lightning, considerable anthropological issues are at stake.

MARGARET Mead and Samoa, a new Australian film made for television, is the latest engagement in a controversy which began in 1983 with the publication of Derek Freeman's book, *Margaret Mead and Samoa: The Making and Unmaking of an Anthropological Myth* (Harvard University Press). The film has been shown in Australia, the United States, Denmark and Sweden; it is presented not just as a journalistic review, but as a contribution to the debate in its own right, a claim which Derek Freeman endorses in person. Indeed the promise is that matters will be settled once and for all — the film opens with the dramatic announcement that the issues in contention "will be resolved by startling new evidence presented in this programme".

But what is all the fuss about? Why did a critical book on Samoan ethnography stir up such excitement? The public has been led to believe that if Mead is shown to be wrong about adolescent girls in Samoa, this will have decisive implications for debates about how far our capacities and characters are shaped by experience, especially childhood experience, and how far they are fixed by heredity. These are some of the greatest issues which have exercised social scientists in the twentieth century. In the manner of the cinematic genre, however, let us backtrack and recall the story so far...

Mead's study of Samoan adolescent girls was carried out in 1925, when she was 23 years old. It was reported over 60 years ago in *Coming of Age in Samoa* (William Morrow, 1928), a short book written in popular style. At her publisher's suggestion, Mead added a final chapter which drew a moral from Samoa for educationalists in the United States, and her book became a hugely and enduringly successful best-seller. She also wrote a sober ethnography of Samoa, *Social Organization of Manu'a*, which was published by the Bishop Museum in Hawaii, and together with various collaborators she later carried out more sophisticated studies of other Pacific communities.

Nevertheless it is this famous apprentice book which Freeman chose to try and refute more than half a century later, identifying it as the cornerstone of relativist, anti-biological social science.

Mead spent nine months in Samoa, but her main study of 50 girls in one village was carried out in a period of four months,

whether adolescence was necessarily a period of stress and discord, caused by the ineluctable biological processes of puberty, or whether the problems that American adults experienced with teenagers could be put down to specific cultural factors, and so, perhaps, ameliorated or even eliminated by making changes in schools or in family life. According to Mead, adolescent girls in Samoa had no problems of sexual adjustment, enjoying a fulfilling and undemanding love life. They seemed to have an untroubled passage to maturity. There were few sulky, angry adolescent rebels. Adult Samoans were also relaxed, tolerant and genial. Emotions seldom ran high and there were no intense attachments, even within the immediate family. The society was remarkably free of violence and aggression. The conclusion was that the storm and stress of American adolescence was a culturally specific phenomenon, and had to do with American attitudes to sex and to competition, and to the intense pressures of family life.

Freeman disagrees with most of Mead's ethnographic generalizations. He says, for example, that there is strong mother-child bonding in Samoa, that parental discipline is severe, and that there is a high level of general aggression and violence. Adolescent girls do not enjoy sexual freedom; the Samoans put a premium on virginity, but rape is common, and because of the emphasis on chastity a girl may actually be forced to marry her assailant.

The research for Freeman's critique of *Coming of Age in Samoa* was begun in the 1940s, and his own book was substantially completed by the 1960s. Unfortunately it appeared only some two decades later, several years after Mead's death. But although we are denied Mead's response, a number of regional specialists have assessed Freeman's book in some detail.

Did he successfully refute Mead's conclusions? The consensus among anthropologists is that both Mead and Freeman over-generalized in characterizing the 'ethos' of Samoan culture; in any case,

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Duped? Margaret Mead in 1958.

a very short time by modern fieldwork standards (and her work was badly disrupted by a hurricane). Yet her research was not without merit, despite some obvious shortcomings. She became a confidante of the village girls, and carefully recorded their activities and conversations; she applied various psychological tests; and she systematically collected biographical information.

The main lines of Mead's Samoan study are familiar to generations of readers, including millions of college students in the United States. The central issue was

Cornell Capa

defining national character is a hopelessly subjective and impressionistic project, which most contemporary anthropologists have abandoned. The experience of adolescence in Samoa is a different matter, however, and it should be possible to collect accurate information on such questions as the punishment of children, sexual crime, courtship procedures and other matters on which there has been fundamental disagreement. Yet on issues of this kind, Freeman's own data are by no means uncontroversial. For example, he and his wife carried out a 'virgin census', which established the sexual purity of Samoan adolescents. He does not explain how this was done in a community famous, on his own testimony, for its teasing of investigators.

Freeman is also often uncritical in his use of sources. His statistics on criminal violence in Samoa, for instance, are derived from a mixture of reports from different periods and of diverse provenance. He uses them to construct 'rates' and then makes unsystematic comparisons with juvenile delinquency in American and British cities. (Oddly enough, his main comparative source on youth delinquency is an old study by the notorious fraud Cyril Burt.)

What, then, does the film add to the controversy? The central figure is Freeman himself. He is shown in his garden in Canberra, padding through the woods in gloves, driving what seems to be an armoured personnel carrier through quiet suburban streets, wearing funny woollen hats, dressed as a Samoan and as a naval officer — all the while pressing his charges against Mead and her defenders.

Leg-pulling

The main thrust of the film is an endorsement of Freeman's account of how Mead went wrong. Freeman has always claimed that Mead was systematically misled by her Samoan informants. In the film a young American anthropologist testifies that Samoans do go in a great deal for leg-pulling. An elderly Samoan chief then reiterates the point that Samoan girls are notorious teasers. Finally, an 80-year-old Samoan lady appears on the screen. She is identified as one of Margaret Mead's informants from 1925, and a voice-over tells us that "her account will settle once and for all the controversy. . . ." — yes, she says, yes we lied to her, "we just lied and lied".

Epimenides the Cretan who said that all Cretans are liars posed a famous problem for logicians. Samoans who say that all Samoans are liars discredit those who believe anything they may say. Surely Professor Freeman cannot believe that the testimony of a confessed liar will finally persuade his colleagues that Mead's account of Samoa was wrong?

What of the wider issues? Probably, the

general view among anthropologists is that we are very plastic creatures, and that there is considerable culturally induced variation in personality, values and life strategies. But this 'relativist' orthodoxy has always had to contend with a countervailing search for a bed-rock of 'human nature', even of 'nature', that is shared by all human beings, perhaps all primates. Freeman hopes to establish general propositions about human nature; but he is cagey about whether he expects these to be of the sort proposed by Konrad Lorenz, by E.O. Wilson or even by Sigmund Freud.

Yet, although the battle-lines are clearly drawn, certainly by Freeman and by the media, the debate seems in a curious way to pass them by. Even if Freeman's observations are accurate, and Mead's are mistaken, this would not greatly affect our contemporary view of the adolescent experience. Freeman has not tried to show that ethology or social biology offer a more powerful theory of adolescence than any other on offer. He himself quotes the conclusion of H. Katchadourian (*The Biology of Adolescence*; W.H. Freeman, 1977) that "research on ordinary adolescents has generally failed to substantiate claims of the inevitability and universality of adolescent stress", a judgement which would have discomfited neither Mead nor her supervisor, Franz Boas, half a century ago.

Freeman's alternative claim — which he makes in the name of Karl Popper — is that he has refuted Mead. By disconfirming her Samoan findings, he has kicked away the essential crutch of her cultural relativism. This is to assume that Mead's study of Samoan adolescents, carried out 60 or so years ago, was and remains the crucial experiment for the cultural relativists. The claim has only to be formulated in this straightforward way for its flaws to be apparent.

There is therefore little in the view (fostered by Freeman, and taken for granted in this film) that the Freeman-Mead debate is a crucial round in the battle between cultural relativism and a biologically grounded, scientific, universal view of man. Nor are there serious grounds for the claim, supported by some conservatives, that Freeman has undermined the basis for liberal educational philosophies. Nevertheless, the debate *has* had a special resonance within anthropology, for it has fed the contemporary unease about ethnographic fieldwork and writing.

Doubts on this score trouble anthropologists more than any substantive questions today, for they have invested a great deal in the credibility of their characteristic research methods. It is often said that ethnographic fieldwork is to anthropology what the blood of the martyrs is to

the Church. Many leading anthropologists prefer to call themselves ethnographers, and practically every move an anthropologist makes gains in respectability if it is called 'ethnography'. One goes into the field 'to do ethnography' and comes home 'to write an ethnography', and most professionals firmly believe that the better ethnographies will always be of more enduring value than even the most ingenious anthropological theorizing.

Participant observation

A revolutionary new way in which to do ethnography, participant observation, was introduced shortly before Margaret Mead sailed to Samoa and quickly became the central procedure of modern anthropology. Earlier methods of data collection had been drawn from other sciences. For example, the first professional — scientific British ethnographic expedition, the Cambridge University Expedition to the Torres Strait, in 1898, was conducted by natural historians in the manner of a zoological field study. The natives were observed and their responses tested; and although they were certainly allowed to speak, this was only in answer to scientific interrogation. During the First World War, a Polish scientist at the London School of Economics, Bronislaw Malinowski, spent two years on a small island in Melanesia and invented the modern form of ethnographic field enquiry. Malinowski insisted that the observer had to understand the native language and appreciate the actor's point of view in order to assess what he was told, to grasp the difference between what people said and what they did. Customs made sense in context, actions had meaning, people guessed at each other's intentions and adjusted their own behaviour accordingly in order to achieve goals which might be hidden, contradictory, culturally specific and situationally variable. The observer had to come off the verandah (as he said, in what became a famous phrase), because distance lent only the illusion of objectivity, and prevented genuine understanding of what was going on.

Ethnographic fieldwork on the Malinowski model has become one of the main research strategies in the human sciences, and its insistence on the context of action has influenced the study of other primates too, notably by way of the functionalist field studies of African apes initiated by Irvén DeVore in the 1950s. But notwithstanding its successes, it is evident that the move from the neutral, precise and systematic methods of the Torres Strait Expedition to the personal, messy and home-made methods of a Malinowski has its costs.

If the observer must participate in order to learn, then appeals to the authority of personal experience and to unique insights become uncomfortably prominent.

Obviously, it is not going to be easy to control for observer bias. So how can the reader judge between competitive accounts of a particular culture? These methodological questions troubled many anthropologists who did not take the substantive issues in the Freeman-Mead controversy very seriously. Freeman claimed that he could refute Mead by opposing his observations to hers, but this has turned out to be a difficult task.

To begin with, there have been other attempts to test Mead's findings, which have reached less severe conclusions. Lowell Holmes, an anthropologist who went to Mead's Samoan village 30 years after her study, specifically in order to reassess her work, concluded that her report was substantially accurate (*Quest for the Real Samoa: The Mead/Freeman Controversy and Beyond*; Bergin and Garvey, 1987). In the film, Freeman tries to show that Holmes is biased in favour of Mead for personal reasons, but does not challenge Holmes's findings in a systematic way. Other psychologists and anthropologists who have done research in Samoa are divided in their judgements of her work. (For examples of the various views see "Speaking in the Name of the Real", *American Anthropologist*, Vol. 85, 1983, and Holmes's review of relevant studies in Chapters 8 and 9 of his *Quest for the Real Samoa*.)

But even if Freeman's ethnography is reliable, this does not necessarily demonstrate that Mead got it wrong. She worked in another part of Samoa (under a different colonial regime, with different missionary influences and with a different economic base), and she was working 20 years — and a world war — before Freeman began his own field studies. It may be that both reported what they saw quite accurately, but that they saw different things because things were really different. Alternatively, they may have accurately reported the divergent perceptions of two different segments of the Samoan population. It is perfectly possible that Samoans had two conflicting theories about adolescence, one put about by adults, the other an unofficial, underground ideology of the young people themselves. Freeman may be presenting the point of view of respectable elders, while Mead (who participated mainly in the lives of the adolescent girls) may be generalizing from what was an informal sub-culture.

According to a body of opinion within anthropology, uncertainties of this kind are inevitable and cannot be resolved. If action, even perception, is culturally conditioned, then this applies not only to the subject of study but to the anthropologist as well. No observation is neutral; no observer can free him or herself from the constraints imposed by culture, status and life history. If observers do agree, then



In the field: Derek Freeman in Samoa, 1946.

this is probably because they share a bias. In his book *Orientalism* (Pantheon, 1978), Edward Said has argued that 'Orientalists' constructed an Oriental world to suit the purposes of the Occident.

The Freeman-Mead controversy has therefore fed a 'reflexive' tendency in ethnographic enquiry. The observer should be conscious of the contingent nature of research, and the final text, the ethnography itself, should not mimic scientific presentations but should be experimental, multi-vocal, ironical. Attention should be paid to the circumstances which produced the classic ethnographies, and to the rhetorical tricks which are used to persuade the reader of the ethnographer's authority. (See *Writing Culture* edited by James Clifford and George E. Marcus; University of California Press, 1986. And George E. Marcus and Michael M. J. Fischer's *Anthropology as Culture Critique: An Experimental Moment in the Human Sciences*; University of Chicago Press, 1986.)

This movement drew inspiration from contemporary critical philosophies, particularly those of Foucault and Derrida, and came to constitute a 'post-modernist' school within anthropology, as in other social sciences. One relevant development was an ethnographic sociology of science, which began from Thomas Kuhn's proposition that scientific paradigms had many of the characteristics of ideologies. Scientists were studied as though they were exotic tribesmen. The intrepid explorers discovered that the practice of laboratory research bears little

relation to the methodological accounts which figure in the textbooks, and which preface reports of findings in scientific papers. The conclusion was that scientific discoveries are constructed and established by social processes; that scientific consensus is a function of academic politics. (See Steve Woolgar's *Science: The Very Idea*; Routledge, 1988. And Bruno Latour's *Science in Action*; Open University Press, 1987.)

Post-modernists in anthropology are acutely alert to the importance of disciplinary politics, which play such a part in their theories. Accordingly, they organized effectively within the United States, where the entrenched cultural relativism provided fertile ground for their doctrines. Elsewhere their influence has been patchy at best. Even within American anthropology there has been a spirited and increasingly powerful counter-reaction. (See P. Steven Sangren, "Rhetoric and the Authority of Ethnography" *Current Anthropology* 29:3, 405-435; 1988.)

Yet even if it has only a brief vogue, the post-modernist challenge has already had a significant impact. Those who are formulating a response would not, on the whole, propose to reinstate the fiction of an objective observer who can bear witness to another culture 'as it really is'. Nor do they deny that research is a social process, and that ethnographies are social constructs. But this does not mean that there can be no firm standards. There is a discipline inherent in the process of ethnographic discovery. The observer and the subject do not simply circle round each other in mutual incomprehension — rather, they engage in a dialogue in which tentative agreement may be reached about actions and their meanings.

The ethnography which results is not a work of art, to be read more or less perceptively. It is a contribution to a debate about specific social processes and their significance. An ethnography will be taken up by a community of experts (including natives) who can call upon their experience of life in particular societies, and who will refer to a body of published observations and interpretations written from different perspectives and within a variety of disciplinary traditions. There is, then, an authority in ethnography, one which is not necessarily embodied in any one account, but which is emergent in the processes of expert research, comparison, evaluation and debate. Derek Freeman's crusading style does a disservice to rational academic discourse, but his dogged polemics have at least made many people aware that there are considerable issues at stake in contemporary anthropological research. □

Adam Kuper is Professor in the Department of Human Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH, UK, and editor of *Current Anthropology*.

Heliocentric tangents

SIR—The heliocentric hypothesis, so ably championed by Copernicus and Galileo¹, is authoritatively said to have originated with Aristarchus of Samos in the third century BC. *Sand-Reckoner*, written by Aristarchus's younger contemporary Archimedes before 216 BC², attributed to Aristarchus a book containing the hypotheses "that the fixed stars and the sun remain unmoved, [and] that the earth revolves about the sun in the circumference of a circle, the sun lying in the middle of the orbit . . .". Aristarchus's (lost) book is thought to have "clearly . . . also included some kind of geometrical proof"³.

Aristarchus had also produced a treatise *On the sizes and distances of the Sun and Moon*, which has survived intact. Its "excellent" methodology confirms that Aristarchus's (probably later) heliocentric hypothesis was similarly "not irresponsible" but rather the work of a "conscientious astronomer"².

Nicholas Copernicus's *De Revolutionibus* (1543) acknowledged Aristarchus but not his heliocentric hypothesis⁴; however, it seems certain that Copernicus was also acquainted with the latter. For example, his original manuscript had referred to the opinion of Aristarchus on the movement of the Earth, but this reference was subsequently "suppressed"⁵ or "scored out"⁶. Moreover, in relating the views of Philolaus, Heracleides and Ecphantus on the question of movement of the Earth, Copernicus's Preface quoted from *De Placitis Philosophorum* of pseudo-Plutarch, a work in which may also be found: "Aristarchus places the Sun among the fixed stars, and holds that the Earth moves around the Sun's circle"⁴. And *De Revolutionibus* (IV, 32) cites Archimedes' *Measurement of the Circle*, a treatise commonly found in the company of *Sand-Reckoner*⁷.

Copernicus's unquestionably pivotal contribution to astronomy was his grand revival of the heliocentric hypothesis as a systematic planetary theory¹. But in order to fit theory to observations, Copernicus had retained the geometric devices used by Ptolemy (the deferent, epicycle and excentric), and had referred details of planetary movements not to the Sun but rather to the centre of the Earth's orbit. Because of technical and other difficulties with the copernican system, the astronomer Tycho Brahe (1546–1601) rejected it. Brahe had compiled an unrivalled set of observations, which he thought would demonstrate that the Sun and Moon travel around the Earth while the other planets travel round the Sun⁸.

After Brahe's death, Johannes Kepler (1571–1630) invested years in the analysis of Brahe's data, culminating in the derivation of Kepler's three laws of planetary

motion. These provided a precise and enduring mathematical characterization of the heliocentric hypothesis, thus serving to support the position of Copernicus while ironically refuting that of Brahe.

STUART HALE SHAKMAN

2269 Chestnut St, Apt 115,
San Francisco, California 94123, USA.

1. *Nature* **337**, 101 (1989).
2. Sarton, G. *A History of Science: Hellenistic Science and Culture in the Last Three Centuries BC*, 54–57 (Harvard University, Cambridge, 1959).
3. Heath, T. *Aristarchus of Samos*, 301–302 (Clarendon, Oxford, 1913).
4. Armitage, A. *Copernicus* (Allen & Unwin, London, 1938).
5. Heath, T. *The Works of Archimedes* xxiv–xxvi (Dover, New York, 1912).
6. Armitage, A. *Sun Thou Stand Still* **140**, 169–175 (Sigma, London, 1947).

CFC photolysis

SIR—It is certainly possible (*Nature* **338**, 100; 1989) that "the great Antarctic ozone hole is a place where [CFCs and their products are] washed out on to the ice-cap". But this is not the rate-limiting factor in the very long tropospheric lifetimes of CFCs. The rate-limiting factor is simply the rate at which CFCs are carried up into the stratosphere to be photolysed. The sites of this photolysis are the middle and high stratosphere outside the polar night, not the ozone hole; and all the evidence points to the conclusion that this rate is controlled by atmospheric dynamics and that it is not particularly sensitive to details of how and where photolysed material returns to the troposphere. If anything, the throughput could be a little weaker in strong ozone-hole years.

M. E. MCINTYRE

University of Cambridge,
Department of Applied Mathematics
and Theoretical Physics,
Silver Street, Cambridge CB3 9EW, UK

Status of science

SIR—I agree with the gist of your leading article "Science squeezed" (*Nature* **338**, 1; 1989) on the shortage of young researchers in Britain. Research should be shown to be an honoured as well as an honourable profession, but the salaries paid by the state are surely indicative of the public's view of the comparative standing of the profession, as we have recently seen in the case of nurses.

I would like to take hospital physicists as an example of the standing of scientists. They have recently accepted, under duress, a pay rise of only 5.5 per cent, whereas the medical profession has been awarded a rise of more than 8 per cent. This is a fairly typical experience for scientists employed by the National Health Service and the story could probably be repeated in other

countries. I realize that hospital physicists are not predominantly engaged in research, but the country is tragically short of physicists in many areas. It is not surprising that there is now a substantial shortage of hospital physicists, yet the British public was only recently outraged when the calibration of an instrument used for radiotherapy in a hospital was wrongly set.

Lord Zuckerman, in *Scientific American* (September 1988, page 106), said that the most important element in the role of the scientific adviser to the chief executive (president or prime minister), is to "advise . . . on whether or not the country's educational institutions are turning out enough adequately trained manpower to fill the jobs that determine the well-being of the nation. A president, . . . has to feel confident that everything that can be done to satisfy this objective is being done, given the resources that can be made available." There is no evidence that this is happening in Britain.

PETER N. CAMPBELL

Department of Biochemistry,
University College London,
London WC1E 6BT, UK

Reprint research

SIR—Previous correspondents have argued for and against the reprint system, but have provided very little information about the pattern of reprint requests. You may therefore be interested in the sources of requests for reprints of my paper "Population dynamics of starlings in New Zealand" (*N.Z. J. Ecol.* **4**, 65–72; 1981). Starlings, being cosmopolitan, should interest all countries; publication yielded a nice collection of stamps and the following tally: United States 24, Canada 4, Finland 4, France 4, Japan 4, Hungary 3, Spain 3, Sweden 3, Australia 2, Belgium 2, Czechoslovakia 2, East Germany 2, West Germany 2, Israel 2, Poland 2, Alaska 1, Denmark 1, Italy 1, Latvia 1, Mexico 1, South Africa 1, Soviet Union 1, United Kingdom 1.

Given these data, one may speculate. Has the Soviet Union 24 times as many Xerox machines as the United States, or has the United States 24 times as many starlings? What does the United States do with all these reprints when it is said that Americans never quote overseas literature? If only one in 24 Soviets reads English, what do the English read? The intense rivalry between East and West Germany is mirrored in the exactly similar number of reprint requests, but why the balance between Latvia and Mexico? Finally, look at New Zealand: 3 million people, and none could afford the stamp.

JOHN E. C. FLUX

Ecology Division, DSIR,
PO Box 30379,
Lower Hutt,
New Zealand

Complicated problems not yet soluble

While physics may remain the art of representing difficult problems as simple, even the simplest complicated problems appear to have the practitioners bemused.

EVERYBODY knows by now that physics is merely sleight of hand. Very few problems of the real world are exactly soluble, and then only because it has been possible to simulate the real by a much simpler problem. The whole of kinetic theory is nothing more than an account of the motion of billiard balls, or elaborations thereof. The vibration of a turbine blade in a jet engine is described in terms of what is essentially the motion of a pendulum, as if it were just another example of simple harmonic motion.

That it may be far from simple to tell from first principles what are the frequencies of vibration of a turbine blade only serves to illustrate the point: the most widely used techniques are in at least two senses approximate — the predicted frequencies of vibration will be more or less in error and, even more significant, the only certainty is that any finite list of vibration frequencies must be incomplete.

This state of affairs is widely if not ostentatiously acknowledged. If challenged, people will readily turn the question against those who ask it, saying that the simulation of so many complicated by so few simple problems is a measure of the sophistication of their craft. But the business of chaos is a reminder that not everything is dealt with simply.

Even apparently simple dynamical systems capable of simple harmonic motion will behave unpredictably if, for example, there are forces which are not proportional to the displacement or some time-derivative of it. But while such a motion may be complicated, the same cannot be said of the underlying system, which has only two degrees of freedom (one space coordinate and time). That is the dark side of the claim that physics is the craft of making complicated problems simple: the search for simplicity blinded people to complexity.

The study of truly complicated systems is another matter, but the past few years have seen their surreptitious accumulation. These are the problems with many degrees of freedom in which the multiplicity is of the essence.

A simple regular idealized crystal lattice is a good illustration. If there are N atoms at the lattice points, $3N$ coordinates will be needed to specify their displacements from their equilibrium positions and there will be $3N$ frequencies of vibration which, once calculated, will describe the vibration of the crystal as if it were the vibration

of any one of $3N$ oscillators.

But if the lattice points are occupied by quantum spins (as in, say, a ferromagnet), and if the problem is to calculate the response of the system to an external field, there is no such route to simplicity. The state of all spins at each instant is essential to an understanding of how the system as a whole behaves. Ways of approximating to an understanding by what are called, for example, 'mean-field' approximations, may yield gross measures of, say, magnetization — but then only well away from the Curie temperature. Subtle features of such a system, say the speed of motion of the boundaries between magnetized domains, for example, remain incalculable.

The recently accumulated examples of this kind keep many people awake at night. Neural networks are a fashionable example, where the instantaneous state of all the neurons in an interconnected network is crucial to an understanding of how one state of the network switches to another. But that is merely one reason for remarking that, against the prevailing grain of physics, there appears from the literature to be a small group of people working away at systems which, by having many degrees of freedom, are inherently complicated, but which are nevertheless tractable.

There is, for example, the case of the proposed use of arrays of interconnected superconducting Josephson junctions as means of analysing the frequency of millimetre-wave signals. Kurt Wiesenfeld and Peter Hadley from the Georgia Institute of Technology have now given a fascinating account of this system (*Phys. Rev. Lett.* **62**, 1335; 1989). Those interested will find an earlier preliminary account (with M.R. Beasley) helpful in fixing ideas, as the saying goes (*Appl. Phys. Lett.* **52**, 1619; 1988).

A Josephson junction is a two-dimensional superconducting circuit (on a chip) whose dimensions are such that the wavefunctions of the circulating electrons have dimensions comparable with those of the whole device. One consequence is that the electrical behaviour of a single junction in an array is determined by the phase difference between its electronic wavefunction and that of neighbouring wavefunction. Josephson junctions, in other words, are a means of making quantum properties macroscopically measurable. Arrays can be fabricated with a difficulty that may

seem child's play to those who make fortunes in the semiconductor industry.

But to make such arrays function as advertised in the millimetre-wave business, all the junctions in an array must oscillate together, in phase with one another. The difficulty is that the equation that determines the oscillation of a single junction in an array is inherently non-linear. (Apart from a few constants, the quantity representing the restoring force in simple harmonic motion appears as the trigonometric sine of that quantity, and there is no presumption that the equivalent of the restoring force is small, because the argument of the trigonometric sine can be anywhere between 0 and 2π .) The consequence is the sequence of *ad hoc* arguments by which the authors reach their interesting conclusion.

This is how it goes. It is easy enough to show that the condition in which all the junctions in an array oscillate as one, with no phase differences between them, is a solution of the equations; that is why arrays of Josephson junctions were advocated as local oscillators in the first place. The practical question is whether such an oscillating state will be stable. Bereft of more explicit alternatives, the authors go through a counting exercise, adding up the number of oscillatory states of a Josephson array in which the phases will be out of synch.

The obvious practical question is whether the discriminatory power of a local oscillator built on lines like this will increase as the number of junctions in the array increases. Naturally, there must be a tendency in that direction, but there is also a countervailing influence. Put crudely, each out-of-synch state of oscillation is potentially a state into which the intended stable state may be kicked by random noise — and the out-of-synch states are crowded ever more closely together (in what is known as phase space) as the number of elements increases. A further consequence, demonstrated only by numerical simulation, is that an increase in the number of elements in an array quickly decreases (virtually to zero) the time an array will spend in any particular oscillatory state.

On the face of things, that would seem to spell the end of Josephson arrays as millimetre-wave detectors, but really it is merely a signal that much more needs to be learned of a complicated simple system.

John Maddox

Defective viruses to blame?

Robin A. Weiss

THREE new papers on an acquired immune deficiency syndrome in mice caused by a strain of murine leukaemia virus suggest that a defective genome is responsible for the immunodeficiency. Janet Hartley, Sandy Morse and colleagues¹ at the National Institutes of Health have found an unusual pattern of genetic susceptibility to murine AIDS consistent with a defective genome being carried by different replication-competent 'helper' viruses. This has been followed up by the molecular identification, also by Hartley and colleagues², of the defective virus. And an apparently identical genome has been independently identified by Paul Jolicoeur and colleagues in Montreal, as they report on page 505 of this issue³. After cloning of the defective virus, which retains *gag* sequences in its 4.8-kilobase genome, and rescue by non-pathogenic helper viruses, inoculation into mice leads to the induction of AIDS^{2,3}. These findings supplement that from Jim Mullins's group⁴ which shows that in cats, immunodeficiency can be caused by a defective feline leukaemia virus.

Many classes of animal RNA virus accumulate defective variants when propagated serially at high multiplicities of infection. This was first observed by von Magnus with influenza virus⁵. Such defective viruses frequently interfere with the replication of full-length, non-defective genomes and they may play a role in pathogenesis and persistent infection⁶. Defective retroviruses, however, do not typically interfere with their helper viruses and are not simply truncated forms of the competent virus; they contain recombinant viral or cellular sequences that can directly influence the pathogenesis of the virus population.

Retroviral oncogenes

The notion that defective variants of retroviruses cause specific disease was raised more than 25 years ago, when Hanafusa *et al.*⁷ showed that the Bryan strain of Rous sarcoma virus was defective. This classical study led eventually to the identification of oncogenes derived from cellular genes, usually by insertion at the expense of viral genes, thereby rendering the viral genome replication-defective. Nearly all retroviral oncogenes are carried in defective viruses requiring helper viruses for transmission.

That defective retroviruses can carry 'scrambled' viral genes determining new disease patterns, rather than cellular genes, was first seen with the spleen-focus-forming virus (SFFV) component of Friend erythroid leukaemia virus. The defective SFFV genome encodes an incomplete

retroviral *env* gene derived from exogenous, 'ecotropic' and endogenous, 'xenotropic' sequences. This new form of glycoprotein probably interacts with specific 'dual-tropic' receptors on erythroid precursor cells to trigger their proliferation⁸. As the term 'viral oncogene' is usually restricted to gene sequences of mainly cellular origin, and of course pertains to those genes inducing neoplastic transformation, we need a new term for retroviral genes of viral origin specifying particular disease forms — I would offer as a suggestion 'pathogenes' with path as the generic equivalent to onc.

The SFFV gene determining erythroleukaemia is derived from *env* sequences⁸, as is the gene inducing feline AIDS in defective feline leukaemia virus⁴. A recent paper by Poss *et al.*⁹ indicates that the property of the *env* protein of the defective feline AIDS virus which makes it cytopathic for T cells lies in its post-translational glycosylation pattern. With murine AIDS, however, it appears that the functional path gene in the defective virus is derived from the *gag* gene. Aziz, Hanna and Jolicoeur³ have sequenced the 4.8-kilobase genome and found four open reading frames, two corresponding to portions of *gag*, one to part of *pol* and one to part of *pol-env*.

The p12 region of the largest open reading frame (586 amino acids) has only 40–50-per-cent identity with Gag p12 of other murine retroviruses, and it is this product that the authors suspect is responsible for inducing AIDS. Chattopadhyay *et al.*² also implicate a *gag*-related product because Gag antisera including anti-p12, but not anti-Pol and anti-Env sera, immunoprecipitate a 60-kilodalton protein from a murine cell line carrying the defective genome in the absence of helper virus.

The study of defective murine and feline leukaemia viruses causing immune deficiency syndromes should enhance our understanding of the various ways retroviruses induce disease. Being oncoviruses rather than lentiviruses and therefore less closely related to the human immunodeficiency virus (HIV), their use in screening anti-viral drugs is doubtful; if one wishes to use an oncovirus for this purpose, Friend virus induces disease in mice more quickly with a more quantifiable endpoint.

Aziz *et al.*³ cite striking similarities of murine AIDS to human AIDS, though there are striking differences too. The disease was first described as lymphoma¹⁰, owing to what is now believed to be a polyclonal B-cell hyperplasia rapidly induced by the virus. A detailed study¹¹ of

the syndrome showed that a profound suppression of humoral and cellular immunity soon follows the lymphoproliferative phase. Both the B-cell hyperplasia and the immune suppression seem to be T-cell dependent, because the virus affects nude mice much less severely¹². However, it is not clear that the virus infects T-helper cells, and the macrophage reservoir and neurotropism characteristic of HIV have not been examined in murine AIDS. In my view, the diseases induced by HIV-related, T-lymphotropic lentiviruses of cats (FIV), cattle (BIV) and monkeys (SIV) will prove to be more proximate models of human AIDS.

Role in human AIDS

Nonetheless, the discovery of defective oncoviruses inducing severe immunosuppression in cats and mice raises the question whether defective HIV genomes might play a role in human AIDS. Replication-competent, molecularly cloned HIV and SIV genomes have not yet been shown to induce AIDS, and even if they do, that might require the generation of defective, variant genomes during the long incubation period between infection and manifestations of disease.

Several HIV laboratories are finding a myriad of viral forms when sequences are amplified directly from infected tissues, rather than selecting for replication-competent viruses in cell culture. Simon Wain-Hobson (personal communication) refers to such HIV populations as 'quasi-species' because they represent greater diversity than might be expected from classical population variability. The mixture of defective oncoviruses and the helper viruses required for their generation and transmission represents a special case of quasi-species genome dynamics. No doubt defective forms of HIV will be carefully scrutinized at the molecular level. □

Robin A. Weiss is at the Chester Beatty Laboratories, Institute of Cancer Research, London SW3 6JB, UK.

- Hartley, J.W., Frederickson, T.N., Yetter, R.A., Makino, M. & Morse, H.C. *J. Virol.* **63**, 1223–1231 (1989).
- Chattopadhyay, S.K., Morse, H.C., Makino, M., Ruscetti, S.K. & Hartley, J.W. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Aziz, D.C., Hanna, Z. & Jolicoeur, P. *Nature* **338**, 505–508 (1989).
- Overbaugh, J., Donahue, P.R., Quackenbush, S.L., Hoover, E.A. & Mullins, J.I. *Science* **239**, 906–910 (1988).
- Von Magnus, P. *Acta path. microbiol. immun. scand.* **28**, 278–293 (1951).
- Huang, A.S. & Baltimore, D. *Nature* **226**, 325–327 (1970).
- Hanafusa, H., Hanafusa, T. & Rubin, H. *Proc. natn. Acad. Sci. U.S.A.* **49**, 572–580 (1963).
- Li, J.-P., Bestwick, R.K., Spiro, C. & Kabat, D. *J. Virol.* **61**, 2782–2792 (1987).
- Poss, M.L., Mullins, J.I. & Hoover, E.A. *J. Virol.* **63**, 189–195 (1989).
- Haas, M. & Reshef, T. *Eur. J. Cancer* **16**, 909–913 (1980).
- Mosier, D.E., Yetter, R.A. & Morse, H.C. *J. exp. Med.* **161**, 766–784 (1985).
- Mosier, D.E., Yetter, R.A. & Morse, H.C. *J. exp. Med.* **165**, 1737–1741 (1987).

EARTHQUAKES

Global perspectives of chaos

Christopher H. Scholz

RECENT developments in nonlinear dynamics have not failed to capture the imagination of geophysicists, whose domain encompasses a great many nonlinear processes. For solid-Earth geophysicists, the most exciting possible applications are to mantle and core convection and, especially, earthquakes. Any segment of the stressed boundary between two plates exhibits a seismic cycle defined by the recurrence of large earthquakes, between which only minor seismic activity occurs. In many cases, this cycle does not seem to be periodic, but the question "is the system chaotic, and if so, in what way?" is yet to be resolved. Condensed matter physicists and geophysicists came together at a recent meeting* to discuss such questions. Each group is still learning the other's discipline, so that what transpired was a preliminary dialogue which nonetheless contains a foretaste of things to come.

The elastic rebound theory of earthquakes, developed by H. F. Reid at the turn of the century, is often inferred to predict the periodic recurrence of large earthquakes, but even in Reid's day it was recognized that the spatial heterogeneity of faults would result in aperiodic behaviour (G. K. Gilbert, *Science* **29**, 121–138; 1909). The seismic cycle can be thought of as a friction-damped oscillator with many spatial degrees of freedom, which is almost a prescription for chaos. As symptoms of this, it is already known that the system abounds in fractal distributions. The Gutenberg–Richter power-law size distribution of earthquakes, as near to universal as any geophysical observation, is one such fractal distribution, and it has also been found that the size distribution of faults, fault topography and even the grain-size distribution of fault gouges are fractal (D. Turcotte, Cornell University; C. Sammis, University of Southern California). Earthquake faults thus have the same fractal nature as other fractures, as discussed in a recent News and Views article by Robert Cahn (*Nature* **338**, 201; 1989).

The seismotectonic system can be regarded as a nested hierarchy of fractals, ranging from topography produced by active faulting down to the material

ground between the fault walls. Although they have learned these geometric facts, geophysicists have only just started to think about the dynamic systems that can give rise to such structures. Any models developed in other fields of nonlinear science, such as those employing cellular automata or renormalization groups (discrete-variable approximations of differential equations), are ripe to be imported to explain the physics of earthquakes.

Although most nonlinear studies to date have been of low-dimensional systems, a very general model of spatially extended dissipative systems has recently

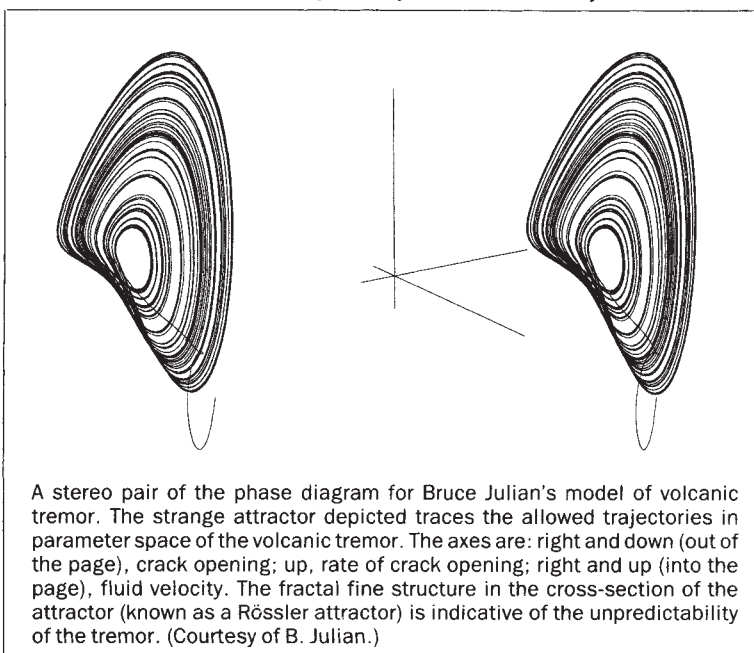
contrast, another cellular-automata model employing simple triggering criteria (T. Hirata, Tsukuba University) has the property that even if started randomly it becomes organized by phase-locking to produce regular large events. Earthquakes may have both of these characteristics. Large earthquakes must roughen the stress distribution on the fault, but in the intervening period, many small earthquakes must smooth it again to set the stage for the next large event.

Whereas the periodicity of the earthquake cycle is a central question for long-term earthquake prediction, short-term prediction depends on identifying the nucleation phase. Diffusion-limited-aggregation and viscous fingering models of nucleation (T. Halsey, University of Chicago; W. Klein, Boston University)

are instructive but not very applicable, as their finely branched structures are unlike earthquake rupturing, which tends to fill the plane. The several forms of a renormalization group model of bundles of parallel fibres under tension produce a chain-reaction nucleation (S. Solla, AT&T Bell Laboratories; W. Newmann, University of California, Los Angeles), but have the unfortunate property of predicting zero strength in the limit of an infinite bundle. This is because the entire bundle fails when the weakest fibre does, owing to the strong nearest-neighbour reloading.

Ropemakers long ago learned to avoid this property by braiding the rope,

which causes friction between strands to be induced by tension on the rope; this causes the problem to become three-dimensional because a broken fibre does not globally lose its load-carrying capacity. Earthquakes, being shear cracks, dissipate energy by friction over their entire surface and so will similarly have a less abrupt nucleation. Nucleation models based on rock friction constitutive laws exhibit scaling that depends on a critical slip distance (J. Dieterich, US Geological Survey, Menlo Park). The critical slip distance is a material (surface) property, which in uniform block-slider models determines the size of the slipping patch at instability (see my paper in *Nature* **336**, 761–763; 1988). However, in spatially extended models in the stable mode, chaotic motion can occur with this friction law, even in the case of spatially uniform parameters (F. Horowitz, Georgia Technical College). This may avoid a problem that seemed implicit in the model: the prediction of a minimum earthquake size.



A stereo pair of the phase diagram for Bruce Julian's model of volcanic tremor. The strange attractor depicted traces the allowed trajectories in parameter space of the volcanic tremor. The axes are: right and down (out of the page), crack opening; up, rate of crack opening; right and up (into the page), fluid velocity. The fractal fine structure in the cross-section of the attractor (known as a Rössler attractor) is indicative of the unpredictability of the tremor. (Courtesy of B. Julian.)

been developed which predicts some of the characteristics of earthquakes (P. Bak, C. Tang & K. Wiesenfeld, *Phys. Rev. A* **38**, 364–374, 1988). This cellular-automata model has the property that it evolves to a self-organized critical state that is characterized spatially by a power-law size distribution of clusters and temporally by '1/f' noise (f is frequency). A simple visualization of it is of a pile of sand, in which the self-organized critical state is the maximum angle of repose, the size distribution of landslides is a power-law, and the sand flow off the pile is 1/f noise. Earthquakes do have this size distribution, and the temporal signature can be checked by the study of earthquake catalogues.

Building further on this development is a model of a network of stick-slipping friction masses connected by springs (J. Langer, University of California, Santa Barbara). This model has the important property that even if it is started in a homogenous state it evolves to chaos. In

* Earthquakes: chaotic or deterministic? Asilomar, California, 12–15 February 1989.

The origin of the fractal topography of faults was addressed by H. Herrmann (Saclay). In a square-grid model subjected to shear forces, crack growth occurs with pervasive branching, resulting in a fractally irregular crack traversing the grid with many side branches. In contrast, the tensile case produced a much more regular structure, with only minor secondary cracks. This result, not likely to be sensitive to the details of the model, explains the much greater complexity of shear to tensile cracks that is well known from both the field and laboratory.

In two other papers not related to earthquakes, modelling predicted chaotic behaviour for diverse phenomena. Volcanic (harmonic) tremor is a nearly continuous vibration that accompanies volcanic eruptions. Treating the source of the vibrations as magma flowing through a compliant crack, B. Julian (US Geological Survey, Menlo Park; see figure) finds that including radiative dissipation introduces a transition to chaos by 'period doubling': as the magma velocity approaches a criti-

cal value, sub-harmonics of the vibration set in until the vibration becomes completely chaotic. And the onset of chaos by way of 'Hopf' and 'pitchfork' bifurcations, splitting of fluid trajectories, has been predicted in models of mantle convection (C. Stewart, Cornell University).

There are sophisticated methods for extracting from a time series of a chaotic process the structure of the 'attractor' (which defines the set of possible states), after which one has a better chance at guessing the system that produced the attractor (J. Sidorowich, University of California, Santa Cruz). But the question for earthquakes is whether geophysical data sets are adequate for this approach. Seismicity catalogues are generally much shorter than the recurrence time of large earthquakes, and it is not at all clear that the attractor for the loading cycle can be obtained from them. □

Christopher H. Scholz is at Lamont-Doherty Geological Observatory, Palisades, New York 10964, USA.

LONG-TERM POTENTIATION

Strengthening the synapses

Charles F. Stevens

ON page 500 of this issue¹, Davies *et al.* provide an answer to one of the important open questions about how long-term potentiation (LTP) works. LTP is a very long lasting increase in synaptic strength that is produced at certain synapses when they are used repeatedly — a sort of microscopic 'practice makes perfect'. This phenomenon has attracted much attention recently because it is thought to be the cellular basis for certain types of memory. Whether the site of increased connection strength is presynaptic or postsynaptic — that is, whether it resides in the sending or the receiving neuron, has been a controversy for some time. The answer Davies *et al.* give is more complicated than most had hoped for: it is both. For the first half-hour after a synapse has been heavily used, the increased strength arises in the presynaptic terminal, and then the processes causing LTP develop in the postsynaptic membrane.

Just how can a synapse get stronger? Understanding the possibilities requires a brief review of how synapses function. Synaptic transmission works by the nerve terminal (the presynaptic element) releasing neurotransmitter through an exocytotic process. In the synapses where LTP has been demonstrated, this is glutamate, which is contained in 40-nm vesicles that are released into the cleft between the pre- and the postsynaptic

cells; the glutamate diffuses rapidly across the cleft and binds to several distinct types of glutamate receptors in the membrane of the target neuron (the postsynaptic element). These receptors open pores through which ions flow. Synaptic strength, then, is determined by the quantity of ions that flow through open pores into the postsynaptic neuron.

A synapse will be stronger if more neurotransmitter is released by the axon

can capture the available neurotransmitter and thus more pores can be opened (the postsynaptic mechanism). An increase in transmitter release might also occur by having each vesicle contain a greater quantity of neurotransmitter. On the postsynaptic side, an increase in strength could also result if each pore remained open for a longer time or if each receptor were to bind its ligand more avidly.

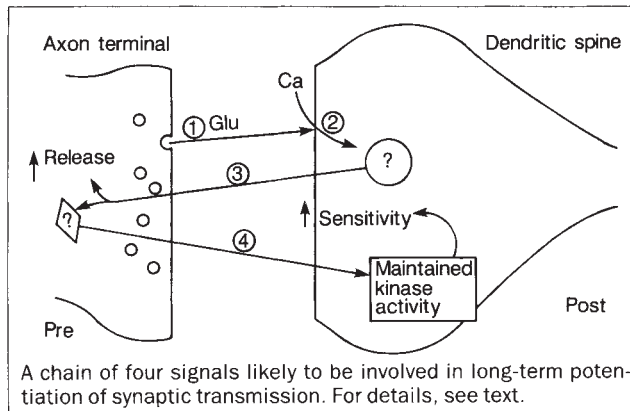
The question, then, has been whether LTP arises from a presynaptic mechanism (more transmitter release) or a postsynaptic mechanism (greater sensitivity of the target membrane). The second alternative, the postsynaptic mechanism, is easiest to believe because it is well established that the crucial signal for triggering LTP (a postsynaptic influx of calcium ions) occurs only in the postsynaptic neuron. Nevertheless, until recently, the weight of evidence favoured the presynaptic mechanism and one was forced to suppose that the triggering signal produced another message that was relayed from the target neuron back to the axon terminal. What Davis *et al.* are proposing is even more complicated.

One good way of detecting a postsynaptic mechanism is to apply neurotransmitter locally to the target neuron and see if it is in fact more sensitive after heavy synaptic use than before. If synaptic strength correlates with postsynaptic sensitivity to neurotransmitter, determined by direct glutamate application, then the mechanism is probably at least partly postsynaptic. If, on the other hand, the postsynaptic membrane is not more sensitive to exogenously applied neurotransmitter, either LTP arises through an increase in transmitter release, or the experiment is inconclusive because the right place on the postsynaptic neuron was not tested.

What Davies *et al.* find is that, even though LTP develops promptly after heavy synaptic use, postsynaptic sensitivity to exogenously applied neurotransmitter (more correctly, to a neurotransmitter analogue) develops slowly over about half an hour. The conclusion, then, is that the mechanism is presynaptic for the first half-hour and that gradually the postsynaptic membrane becomes more sensitive — some signal must pass from the postsynaptic membrane to the axon terminal to produce a transient increase in release. With

time, however, an increased sensitivity of the postsynaptic membrane grows so that LTP is maintained by a postsynaptic mechanism.

Although this mechanism seems complicated, it does fit with other recent observations. Kauer *et al.*² were the first to define clearly two temporal phases of LTP — they discovered a first, transient LTP component that lasts about half an



terminal or if the postsynaptic neuron responds more vigorously to whatever quantity of transmitter is present, or both. The two possibilities that are usually considered are: first, that the axon terminal releases more vesicles, each containing a fixed quantity of neurotransmitter (the presynaptic mechanism for strengthening); or second, that the target postsynaptic membrane contains more receptors than

GEL ELECTROPHORESIS

DNA molecules observed

Ted Richards

hour, the same duration as the presynaptic phase of LTP now defined by Davies *et al.*. This transient component can be evoked in isolation by applying neurotransmitter directly to synapses without stimulating the axon terminals².

More recently, Malinow *et al.*³ have discovered a second way to define transient and maintained phases of LTP. A persistent speculation has been that one fundamental mechanism in the storage of memories is the covalent modification of some (unidentified) proteins by kinases. During an investigation of this hypothesis, Malinow *et al.* found that when kinases are inhibited, the transient phase of LTP is still evoked, but that the maintained phase fails to develop. According to the new results of Davies *et al.*, the transient phase of LTP would be presynaptic, and the maintained component would result postsynaptically from an increased sensitivity of receptors to neurotransmitter resulting from some kinase activity.

All this work has many potential sources for artefacts. If the results are taken at face value, though, the following picture of four inter-related signals emerges (see figure). The first is glutamate that is released from the axon terminal and acts on the postsynaptic membrane to change the postsynaptic voltage and — under the right circumstances — permits an influx of calcium ions. The resultant change in postsynaptic calcium concentration constitutes the second signal and produces, by an unknown mechanism, the third signal that is sent from the postsynaptic cell to the axon terminal where it increases the quantity of glutamate released for each nerve impulse that arrives.

This third signal, in conjunction with some trace of recent heavy use of the axon terminal (calcium again?), causes the fourth signal to be passed from the axon terminal back to the postsynaptic target neuron where it somehow produces a maintained kinase activity that, in turn, results in an increased postsynaptic sensitivity to glutamate.

Why is such a complicated scheme needed? One reason may be that the brain wants to be very careful to store only valid memories, and therefore needs to be sure that parts of the system are not activated accidentally. By having this 'handshaking' scheme, checks are instituted to ensure that the necessary conditions are met for having an appropriate increase in synaptic strength. Whatever the reason, there is clearly still much to learn about the mechanisms involved. □

Charles F. Stevens is in the Section of Molecular Neurobiology, Yale University Medical School, New Haven, Connecticut 06510, USA.

1. Davies, S.N., Lester, R.A.J., Reymann, K.G. & Collingridge, G.L. *Nature* **338**, 500-503 (1989).
2. Kauer, J.A., Malenka, R.C. & Nicoll, R.A. *Nature* **334**, 249-252 (1988).
3. Malinow, R., Madison, D.V. & Tsien, R.W. *Nature* **335**, 820-824 (1988).

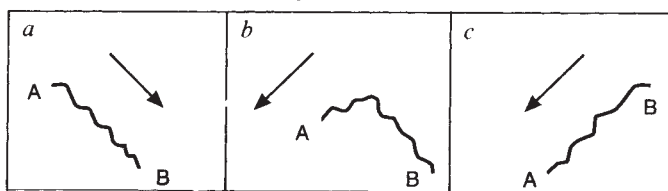
It should be obvious to any reader of *Nature* that gel electrophoresis of DNA has revolutionized molecular biology. It is therefore odd that the precise mechanism whereby DNA molecules, moving under the influence of an electric field in a gel, are separated according to their length, is but poorly understood. The standard description, based on the reptation model of De Gennes, has the molecule snaking down a 'tube' among the gel fibres with the head moving in a direction biased in the direction of the field. This model accounts for the observed inverse relation between mobility and molecular size, which breaks down for long molecules or

toy which consists of a squat helical spring that 'walks' down stairs.

The picture is broadly similar at low-strength fields, but here coils may also start to form in the middle of the extended chain; these may work their way to the trailing end of the chain as the front moves down the field. When they reach the end the trailing end may take off downwards, so forming an inverted 'U' with the chain looped over several obstructions. Eventually the longer end pulls the chain free.

Smith *et al.*³ and Schwartz and Koval² describe experiments in which the motions of real DNA molecules are directly observed. The idea is novel but

Movement of a DNA molecule in an electric field. *a*, The molecule is aligned along the field with end B leading. *b*, The field is reversed and the chain is starting to align in the new direction. The centre of mass has not moved downwards. *c*, The molecule is now aligned in the new direction with end A leading. It can now start to move.



at high electric fields when the mobility is found to be independent of size. It now seems, however, that this model is oversimplified. Deutsch and Madden¹ have now described a computer simulation of the movement, a study which is complemented by experimental observations of real DNA molecules by Schwartz and Koval on page 520 of this issue² and by Smith *et al.* in a paper recently published in *Science*³.

Deutsch and Madden model the DNA molecule by a chain of charged beads and the gel matrix by a rectangular array of obstructions. They set up equations which determine the motion of the beads and solve these numerically on a computer. In this way they obtain a series of 'snapshots' which portray the conformations of typical chains as time progresses. The results unexpectedly show that a chain alternates between a coiled conformation and one in which the chain is extended in the direction of the field. Exactly what happens depends on the field strength.

Starting at the stretched-out phase, Deutsch and Madden find for high-strength fields that the leading end starts to coil up, eating up the rest of the chain as it moves down; this coil becomes entangled with the gel obstructions. Then one end proceeds to move down leaving the other looped over the gel fibres. As this leading end moves in the direction of the field, the loops slip off the obstructions, until eventually the trailing end is freed and the cycle starts again. The whole cyclic motion is reminiscent, to those who have seen it, of the motion of a 'slinky', a

simple. The DNA molecules are first labelled with a fluorescent dye and then allowed to migrate in an electric field in a thin layer of agarose on a microscope slide. When the specimen is illuminated with light of a suitable wavelength, the fluorescing groups reveal the conformation of the DNA chain. The image of the wriggling chain can be recorded on video tape. The motions so recorded by both groups are in good qualitative agreement with the simulations obtained by Deutsch and Madden, and at some variance with standard reptation model.

Deutsch and Madden¹ discuss the assumptions inherent in the reptation model which appear to be violated in their simulations; they also show that the mobility of long chains in high electric fields is independent of the chain length, in agreement with both standard reptation theory and experiment. This effect leads to an experimental impasse whereby any DNA chain longer than about 20 kilobases cannot be resolved using conventional methods. This impasse is much to the chagrin of molecular biologists, who wish to resolve ever larger pieces of DNA, up to the ultimate goal of separating whole eukaryotic chromosomal DNA.

Several groups have found ways out of this impasse by periodically altering the direction of the field. In this way it is now possible to resolve molecules the size of the yeast chromosome, about 2 megabases. Several such methods involve fields oriented at an obtuse angle to each other so that molecules traverse a zig-zag path along the bisector of this angle. The

reptation model provides a schematic picture of how this works (see figure): at each change in field orientation, both ends of the chain move off in the new direction. Only after a certain time, dependent on the length of the chain, is a molecule completely oriented in the new direction; this process must be completed before further net progress is made. Thus longer molecules are retarded more than shorter ones. This mechanism does not explain all the experimental results, particularly those in which the field is periodically reversed.

The simulations of Deutsch and Madden contain some artificial features: they are conducted in two rather than three dimensions (which may not be serious), and the rectangular array of

obstructions cannot correctly mirror the true structure of the gel, which is largely unknown. Further work should ameliorate these difficulties and also suggest what happens when the direction of the field is changed. Nevertheless, it is hoped that these insights and observations will stimulate experimentalists to devise methods of resolving longer and longer DNA chains. □

Ted Richards, formerly at King's College London, is now at 2 Peckarmans Wood, London SE26 6RX, UK.

1. Deutsch, J.M. & Madden, T.L. *J. chem. Phys.* **90**, 2476–2485 (1989).
2. Schwartz, D.C. & Koval, M. *Nature* **338**, 520–522 (1989).
3. Smith, S.B., Aldridge, P.K. & Callis, J.B. *Science* **243**, 203–206 (1989).

CRYSTALLIZATION

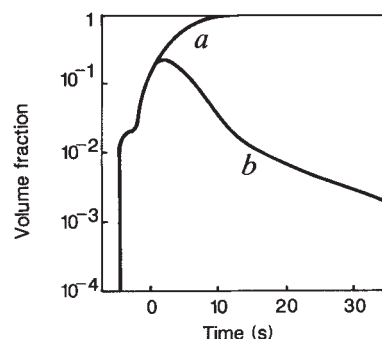
Views of interfacial ephemera

A. Lindsay Greer

QUANTITATIVE measurement of crystallization kinetics in alloy systems has become possible in recent years through the annealing of metallic glasses. In the glasses the atomic mobility is sufficiently low for the rates of crystal nucleation and growth to be measurable and for the sample temperature to remain uniform during the reaction. These are conditions which do not apply in the more common case of crystallization from a liquid. Newly reported work by Sutton *et al.*¹ shows that important information on the structural changes during crystallization of a metallic glass may be lost if the experiment is not conducted in real time during the anneal. The transient interfacial phases suggested in the new work merit further study because of their potential significance in understanding crystallization kinetics not only in glasses, but also in liquids.

In conventional structural studies of the crystallization of metallic glasses, X-ray diffraction experiments are performed at room temperature after annealing. In contrast, Sutton *et al.*¹ use synchrotron radiation to study the transformation *in situ*, with time resolution of 3 ms. The study is of the formation of the body-centred tetragonal phase of NiZr₂ from metallic glass of the same composition. Diffraction patterns from the partially transformed samples cannot be reproduced by superposing the patterns from the initial amorphous phase and from the final NiZr₂, so that the authors postulate there is a precursor phase. It has broad diffraction maxima and is a transient structure existing at the interface between the growing-crystal phase and the amorphous phase. As shown in the figure, the phase is most noticeable in the early stages of the transformation. It is found only during anneals at higher temperature when the transformation is more rapid.

That metallurgical transformations can proceed through intermediate stages (rationalized, for example, by Turnbull's step-entropy rule²) is well appreciated, and is exploited in the conventional practice of quenching and annealing, as in the production of precipitation-hardened alloys. The annealing is stopped before



The time dependence of the total crystalline volume fraction (a) and the volume fraction of intermediate crystalline phase (b) for an anneal of NiZr₂ metallic glass at 680 K (redrawn from ref. 1).

the final equilibrium is reached, to preserve the intermediate microstructure which is technologically useful.

But the possibility of an intermediate phase that exists only during the reaction and that cannot be preserved by cooling is not commonly considered. Such a concept is related to the activated-complex or transition state used in considering the mechanisms and rates of chemical reactions. For crystallization, a first-order transformation, any intermediate state must be associated with the interface between the two phases. One instance where a transient interfacial phase has previously been postulated³ is the explosive crystallization of thin films of an amorphous semiconductor, such as germanium, which is explicable only if

there is a thin layer of metallic liquid, maintained by the release of latent heat, at the interface between the solid amorphous and crystalline phases, both of which have covalent tetrahedral bonding.

The nature and origin of the possible transient phase in the crystallization of Ni–Zr glass must be very different, but likewise it may be significant in analysing crystal growth. The attachment of atoms to a crystal growing in a liquid or amorphous phase may involve diffusive jumps, leading to a maximum, diffusion-limited growth velocity of around 10 m s⁻¹. But measurements of dendrite velocities in undercooled melts led Turnbull² to suggest that for pure metals, the crystal growth rate may be limited only by the collision rate of atoms with the interface. The collision-limited growth rate would then be near the speed of sound, about 200 times the diffusive speed. From pulsed laser experiments, there is firm evidence for each type of growth; collision-limited for pure metals⁴, but diffusion-limited for intermetallic compounds⁵. The transition from one type of growth to the other as the solute content is increased has not yet been satisfactorily analysed.

Sutton *et al.*¹ suggest that the transient phase is a poorly ordered version of the final crystalline structure. Thus there is evidence for growth proceeding in two stages, crystallization followed by chemical ordering, in a manner consistent with the step-entropy rule. Diffusion-limited growth may arise when there is a need for rearrangement of the atomic short-range order at the interface, and just such a process may be revealed by the *in situ* study. The transition between types of growth may be elucidated by studies of the same type on growth of extended elemental solid solutions and of intermetallic compounds with different structures.

The greater fraction of intermediate phase detected at higher temperatures could reflect, as the authors suggest, the higher nucleation frequencies and greater interfacial areas during the transformation. On the other hand, it is possible that it could be associated with the ordering lagging further behind the faster interface. A transition from growth of an ordered phase to growth of a disordered intermediate could affect not only the growth rate, but also the degree of faceting of the crystal through the well-known link with entropy change across the interface⁶. □

A. Lindsay Greer is in the Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK.

1. Sutton, M. *et al. Phys. Rev. Lett.* **62**, 288–291 (1989).
2. Turnbull, D. *Met. Trans.* **12A**, 695–708 (1981).
3. Gilmer, G. H. & Leamy, H. J. in *Laser and Electron Beam Processing of Materials* (eds White, C. W. & Peercy, P. S.) 227–233 (Academic, New York, 1980).
4. MacDonald, C. A. *et al. J. appl. Phys.* **65**, 129–136 (1989).
5. Vitta, S. *et al. Mater. Sci. Eng.* **98**, 105–109 (1988).
6. Jackson, K. A. in *Liquid Metals and Solidification* 174–186 (Am. Soc. Metals, Cleveland, 1958).

EPSTEIN-BARR VIRUS

Chance and felicity

John Galloway

TWENTY-five years ago this week the first report appeared of what came to be known as Epstein-Barr virus (EBV)¹. The finding of a previously unknown virus in tumour cells cultured from a patient with (Burkitt's) lymphoma was a signal event for the Cancer Research Campaign (CRC), then the British Empire Cancer Campaign, and one that the campaign is celebrating next week with an international conference*.

The progress of science is littered with happy coincidences, chance observations and meetings that mark the beginning of successful lines of research. But few have involved as much serendipity in their early stages as EBV. The story began in the 1950s when Denis Burkitt noticed a child in the African teaching hospital where he worked with swelling in all four quadrants of the jaw. It seemed to be neither a tumour nor an infection, but three weeks later he saw exactly the same thing again in another patient. No longer a curiosity, the condition became a medical problem, and in time it was realized to be the commonest childhood cancer in Africa — and indeed that other childhood lymphomas were simply different manifestations of the disease — now called Burkitt's lymphoma.

Burkitt noticed that the disease had a characteristic geographical distribution, clearly related to climate. Climate sugges-

ted biological transmission, possibly an insect vector. He turned out to be right about the climatic connection — his lymphoma had exactly the same geographical distribution as malaria — but not about the insect vector. His work attracted little interest until he gave a lecture in 1961 which was attended by Tony Epstein.



Epstein, Barr and Achong in 1964 (courtesy of M. A. Epstein).

Epstein, persuaded by Burkitt's argument for a biological cause, decided it must be an oncogenic virus². Viruses were known to cause and spread cancers in animals, and, although there was really no evidence, were thought to cause human cancers.

At this point the Campaign paid for Epstein to visit Burkitt in Kampala to obtain biopsy material. In a cell line established in culture from a tumour biopsy, they found, using the electron microscope, a new, large, icosahedral herpes-like virus in the tumour cells. The finding was reported in *The Lancet* and was later named after Epstein and his PhD student Yvonne Barr who, with Bert Achong, was

his co-author. It was fortunate that the laboratory had available thin-section electron microscopy; the virus could not have been detected otherwise as it was inactive in all the biological assays then available.

The serendipity which marked the early work on EBV was repeated later in the Henles' laboratory in Philadelphia, where a chance event revealed that EBV was also the cause of infectious mononucleosis — glandular fever. A technician whose blood was being used as a negative control in serum-antibody tests for EBV infection contracted glandular fever while on holiday and developed a high concentration of antibodies against the virus. A sero-epidemiological prospective study then showed conclusively that EBV was indeed the cause of the disease.

So much for history. What do we know about the virus? Does it really contribute to Burkitt's lymphoma — and nasopharyngeal cancer — as is claimed? Is there any point in developing a vaccine against EBV to prevent these diseases? Why else should EBV be the subject of so much and such intense research?

EBV is a typical herpesvirus, the exemplar of the γ -herpesvirinae. It contains 170 kb of double-stranded DNA, encoding something like 100 proteins, and its genome structure is known in great detail³. It is noteworthy for its complexity and remarkable examples of long range splicing. The large number of proteins it encodes seems to be related to its need to cope with many types of cellular metabolism — it grows in B cells and epithelial

*Epstein-Barr Virus: The First 25 Years, Oxford, 9-13 April 1989.

Mummy deterioration halted by nitrogen atmosphere

MANY museum objects are damaged by microorganisms and insects (see A. David *et al. Science in Egyptology*; Manchester University Press, 1986). We have developed a method to control biological damage and to provide optimum display conditions, and have used a 3,000-year-old Egyptian mummy as a model (see figure). This woman was 40 years old when she died and came from a rich family, shown by the mummification treatment and by a layer of gold over her eyes. The mummy was placed in a hermetically sealed display case and the air replaced by nitrogen at low relative humidity. The combination of low relative humidity (35-40 per cent) and oxygen level (less than 2 per cent) significantly decreases



the biological activity (95 per cent) of microorganisms isolated from the mummy, assessed using carbon-14 tracers. A sealed display case with controlled humidity and a nitrogen atmosphere was designed at the Getty Conservation Institute by Frank Preusser. This is a prototype for showcases to be used in the Egyptian Museum in Cairo. By contrast with common fumigation systems, nitrogen is safe, inexpensive and easily handled. In art object preservation, a nitrogen atmosphere could be an effective means for drying and disinfecting water-damaged materials, and for eliminating insects from museum collections.

Nieves Valentin

Nieves Valentin is visiting the Getty Conservation Institute, Marina del Rey, California 90292, USA, and is at the Instituto de Conservación y Restauración de Bienes Culturales, Madrid, Spain.

cells and can undergo a latent or lytic infection. Since the number of its potential hosts is small (humans and a few primates), its characteristic property of persistence for long periods of time in a latent stage from which it can be rapidly activated is sensible behaviour.

The virus is very common, more than 90 per cent of the world's population is infected. It is ironic that a suggestion of a viral involvement in a not very common cancer, of limited geographical distribution, should have led to the discovery of one of the commonest human viruses. The most interesting property of EBV is that both *in vitro* and *in vivo* it infects and immortalizes human B lymphocytes which then grow indefinitely. (The symptoms of glandular fever represent the cellular immune response to immortalized B cells.) It is the investigation of this property, and the gene regulation that governs latent immortalization and reactivation, that is the basis of EBV research today.

On the face of it, the infection of B cells clearly underlies Burkitt's lymphoma, which is a B-cell tumour, although the malignantly transformed lymphoma cells are in many ways distinct from *in vitro* immortalized B cells. The tumour is clonal both for the cells and for the viral genome, so the virus was probably present in the original B cell that underwent malignant transformation. This seems compelling evidence for a causal malignant transformation between virus and tumour. The argument is strongest if few normal B cells are infected in those who develop the tumour. If, as is generally the case, the proportion is less than 1 per cent, the evidence is very strong indeed. It is not known, however, whether the percentage is so low in African children. Although EBV is almost certainly a contributory cause of Burkitt's lymphoma in the high-incidence areas of Africa it certainly is not a sufficient one (EBV-negative cases of Burkitt's occur all over the world). The effects of malaria on the immune system seem to be an essential factor — the true explanation of Burkitt's original observation of a climatic relation — and the tumour cells also possess a translocation which deregulates expression of the proto-oncogene *myc*. The translocation of *myc* is probably the transforming event. The role of immune surveillance is underlined by recent reports of true EBV-positive Burkitt's lymphoma in AIDS patients in the United States.

As a medical problem, Burkitt's lymphoma is hardly comparable with nasopharyngeal cancer, a disease which is most important in South-East Asia, with an incidence of 10–20 cases per 100,000

population a year. All tumour cells seem to contain virus, but the same reservations hold because nearly everyone has EBV in parts of their oropharynx. It seems that other factors must also be involved, for example, diet.

Following their original support for Epstein in the early 1960s, the CRC continued to support him in his pursuit of an anti-EBV vaccine as well as supporting much other EBV research. Vaccine research has concentrated on the glycoprotein antigen gp340 found on the viral envelope. The next step is to discover whether this molecule will evoke an immune response in man.

From a medical point of view, taking a

QUANTUM ELECTRONICS

Interference rules the waves

Gerhard Fasol

QUANTUM mechanics shows that electrons are waves. On the other hand, to explain conventional transistors, electrons are treated as classical particles propagating by diffusion. Thus there is usually no need to take the quantum-mechanical nature of electrons into account in modelling conventional devices. However, the wave character of electrons is unambiguously demonstrated by various experiments including tunnelling through potential barriers, which would be impenetrable for particles governed by classical physics, interference and diffraction. It is only recently that electron interference phenomena have become important in solid-state physics. Thus proposals for transistors based on quantum interference, as exemplified by an ingenious new design by F. Sols *et al.*¹, are stimulating much interest.

In principle, transistors based on quantum interference are very attractive propositions, because of their very high expected speed. Thus they would be candidates for use in ultrafast computers, high-capacity telephone exchanges or satellite communication systems, for example. Unfortunately, present-day technology restricts such applications to extremely low temperatures (below 1 K). Thus conventional liquid helium cooling is not sufficient — a 'dilution' refrigerator is also needed. A common driving force of research on new types of transistors is to look for operating principles which use phenomena other than diffusion and charge-density modulation, which are both inherently slow. Deformations of wavefunctions and wave interference, on the other hand, are very much faster phenomena.

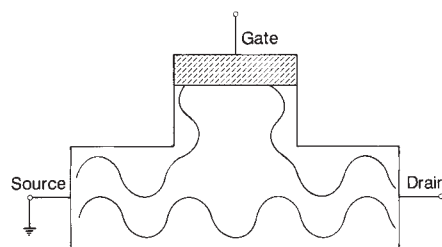
Maybe the most fundamental demonstration of the wave character of electrons is the generation of electron interference patterns, or electron holograms². Electron

shot at producing a vaccine and going to field trials must be the priority for an organization whose *raison d'être* is cancer control. However, the significance of EBV goes far beyond this. It is a particularly valuable and interesting system in which to study virus latency and reactivation, exactly how the virus immortalizes the B cells, and it may shed light on more general aspects of cellular gene expression. Next week's conference should, with luck, bring us closer to understanding these questions. □

John Galloway is head of public relations at the Cancer Research Campaign, 2 Carlton House Terrace, London SW1 5AR, UK.

holography has recently been investigated very thoroughly by Akira Tonomura *et al.*³ at the Hitachi Central Research Laboratory. Their experiment is based on the same principle as optical interference: an electron beam is split into two parts; the two partial waves then follow different paths and are subsequently allowed to interfere. The interference pattern is a consequence of the different phase of the two partial waves. In this way, magnetic flux patterns in superconductors or other structures which change the phase of an electron wave can be investigated.

Interference is well known for light waves: Newton's rings or the holograms used as a security device on credit cards are some well-known examples. A crucial condition for the observation interference phenomena is that the two beams interfering are coherent. This means that there must be a constant phase relationship between the two interfering partial waves over a time span corresponding to the difference in the path length of the two beams. In solids, it is not clear initially whether electron interference phenomena should ever be active, or indeed whether



The novel transistor proposed by Sols *et al.*¹ relies on interference between electron waves passing directly between drain and source and those diffracted via the stub. Applying a voltage to the gate changes the effective length of the stub, so that the waves can be made to interfere constructively (as shown) or destructively.

1. Epstein, M. A., Achong, B. G. & Barr, Y. M. *Lancet* **1**, 702–703 (1964).
2. Epstein, M. A., in *Burkitt's Lymphoma: a Human Cancer Model* (IARC Scient. Publ. No. 60 (1985)).
3. Farrell, P. J. *Adv. viral Onc.* **8**, 103–131 (1989).

experiments can be constructed where they should be observable. This is because in an ordinary semiconductor or metal, electrons collide continually with lattice vibrations, impurities, dislocations and other lattice imperfections. In addition, electrons collide with each other. Thus under normal conditions the coherence length is expected to be much too short to observe any interference phenomena.

But recently it has become clear that elastic scattering (impurity scattering, for example) changes the phase of an electron wave in a reproducible manner. Thus the observation of electron interference is limited only by inelastic electron-electron scattering and by phonon (lattice-vibration) scattering, which can be reduced by cooling the sample to extremely low temperature. The phase coherence length for electrons in GaAs heterojunctions is of the order of 5 μm at 0.1 K, decreasing rapidly as temperature increases (ref. 4; also D. Wharam, personal communication). Thus, using current knowledge, any possible device based on quantum interference of electrons would have to be operated at extremely low temperatures and using dimensions at the limit of today's technology. Nevertheless, solid-state physics has been a source of exciting surprises recently: the discoveries of the quantum Hall effects (see my earlier News and Views article⁵), or high-temperature superconductivity, both rewarded by Nobel Prizes, were not at all expected before their experimental discovery. Therefore we should not have any prejudices in this presently very exciting field.

Transistors (core components occurring millions of times in any electronic equipment) are three terminal devices. In one particular class of transistors there is a current entering at one terminal and leaving at another. A third terminal controls this current: a large change in current through the first two terminals occurs if a small voltage (or a small current) at the third terminal is varied. In an intriguing new suggestion for a quantum interference transistor¹, F. Sols *et al.* propose to use the wave character of electrons for transistor action. The authors investigate theoretically the propagation of electron waves through a T-junction-type waveguide, where a stub of variable effective size is attached to an electron channel. Model calculations show that the transmission coefficient through the channel depends strongly on the size of the stub (see figure). The effective size of the stub can be varied by changing the voltage applied to a gate on the stub. Thus such a device can be used as a transistor.

Tunnelling is another manifestation of the wave character of electrons: materials can pass through thin regions of material classically forbidden to them. M.A. Reed *et al.*⁶ have just demonstrated the operation of a 'quantum resonant tunnelling trans-

sistor' in which the strength of the tunnelling current between two terminals can be modulated strongly by a voltage applied to a third terminal. The essential physics of the effect was recently described in News and Views by Luryi⁷.

Other suggestions of unconventional transistor principles include that by Sakaki⁸, in which the wavefunction of electrons is switched between high and low mobility states — a velocity-modulation transistor. In this device, applying a voltage distorts the wavefunction of the electrons in a layered transistor structure perpendicular to the plane in which electrons are confined. Thus the electrons can be deformed towards the Coulomb scattering centres, reducing their mobility; or they can be pushed away from the scattering centres, increasing their mobility. Such a transistor works by controlling the velocity of electrons. This is expected to be inherently faster than the usual modulation of the charge density.

Another proposal relies on the diffraction of electron waves from surface gratings⁹. In this device electrons are confined to a two-dimensional channel and exposed to a periodic modulation of the

thickness of the well or of a potential. For a modulation of the period a , electrons with wavelength $\lambda = a/z$ (or close to it) will be reflected off the periodic grating. This effect is analogous to the Bragg reflection of light waves or X-rays. Thus the speed of electrons of a particular kinetic-energy range will be strongly affected by the grating. 'Negative differential resistance' can be achieved — a phenomenon whereby the current decreases with applied voltage in certain ranges. In a transistor structure, an applied voltage would change the depth of modulation and thus the velocity of the electron waves. $\square$

Gerhard Fasol is in the Cavendish Laboratory, University of Cambridge, Madingley Road, Cambridge CB3 0HE, UK.

1. Sols, F., Macucci, M., Ravaoli, U. & Hess, K. *Appl. Phys. Lett.* **54**, 350–352 (1989).
2. Gabor, D. *Proc. R. Soc. A* **197**, 454–487 (1949).
3. Tonomura, A. *et al.* *Phys. Rev. Lett.* **54**, 60–62 (1985).
4. Thornton, T.J., Pepper, M., Ahmed, H., Andrews, D. & Davies, G.J. *Phys. Rev. Lett.* **56**, 1198–1200 (1986).
5. Fasol, G. *Nature* **334**, 568–569 (1988).
6. Reed, M.A., Frenley, W.R., Matyi, R.J., Randall, J.N. & Seabaugh, A.C. *Appl. Phys. Lett.* **54**, 1034–1036 (1989).
7. Luryi, S. *Nature* **336**, 515–516 (1988).
8. Sakaki, H. *J. Appl. Phys.* **21**, L381–L383 (1982).
9. Sakaki, H., Wagatsuma, K., Hamasaki, J. & Saito, S. *Thin Solid Films* **36**, 497–501 (1976).

PLANETARY ATMOSPHERES

Impacts giveth and impacts taketh away

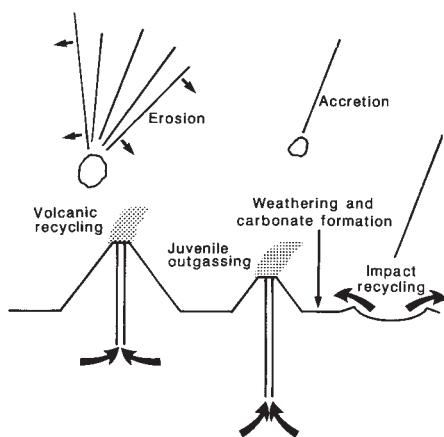
William B. McKinnon

COMETS have often been called upon to deliver life-giving volatiles and organic matter, early in the history of the Solar System, to the presumably barren surfaces of the terrestrial planets. Now, new research by Melosh and Vickery, on page 487 in this issue¹, makes explicit the disquieting notion that bombardment by comets and asteroids can also erode, or even completely strip off, an atmosphere. The authors show by a simple model that Mars, with its relatively low gravity, is particularly susceptible. Working back in time, they find that Mars may have had an atmosphere with a surface pressure more like that of Earth at present. This idea has great scientific appeal as many lines of evidence point to a more clement Mars 3.8 thousand million or more years ago.

The idea that impacts can remove mass from an atmosphere is not new², it is simply not well developed^{3,4}. Melosh and Vickery have modelled the process numerically, but find that its essentials can be described by a simple analytical scheme. The specific mechanism they study is the interaction of the vapour cloud, produced when the impactor strikes the planetary surface, with the atmosphere. Other mechanisms have been studied, such as the shock waves produced by atmospheric passage and the entrainment of air by

high-speed solid ejecta^{3,4}. In both cases, though, workers agree that the amount of atmosphere ejected is not more than a few times the amount intercepted by the projectile on the way in, or by the ejecta on the way out. The beauty of the vapour-cloud model is that much of the impactor's kinetic energy is transformed into vaporized impactor and target. The expanding cloud can then do work on the entire atmosphere in its line of sight.

Two criteria must be met for the expanding vapour cloud to blast the entire atmosphere in its sight to infinity. First, the impact must be fast enough for the vapour to expand at a speed greater than the planet's escape velocity, taking into account the considerable energy required for vaporization. Second, that the vapour mass must exceed the atmospheric mass in question. This is essentially a simple momentum balance. The characteristic sizes of rock impactors that can remove the entire air mass above a plane tangent to the surface are then roughly 3, 13 and 70 kilometres in diameter for Mars, Earth and Venus, respectively. Given these numbers, it is apparent that the atmospheres of all these worlds have been stable against impact erosion over most of geological time. That is, the number of impacts of this size are not great over a



Schematic diagram of the principal sources and sinks for CO_2 in the early history of Mars.

few thousand million years at current cratering rates. The situation was entirely different during the first 500 million years or so of the Solar System, when the impact flux was orders of magnitude higher.

During the era of heavy bombardment, the authors show that the characteristic time for the martian atmosphere to be completely stripped is under 100 million years. Complete stripping is at least theoretically possible, because as the mass of the atmosphere declines, more numerous (and frequent) smaller impactors can erode it. Turning the problem around, Melosh and Vickery integrate backwards in time, undoing the effects of impact erosion. Using the agreed bombardment history for the inner Solar System (see the News and Views article by G. R. Stewart⁵), they show that Mars should have had a relatively thick atmosphere early in its history, barring other significant additions and subtractions. The atmosphere may even have been sufficient to allow running water on the planet's surface.

Impact erosion is clearly an important process in early planetary atmosphere evolution. The challenge ahead is to fit it into the general context. Atmospheres are, after all, not static entities. Earth's oxygen and nitrogen are maintained by living organisms. On Mars the situation is not as clear, but much research has focused on plausible histories for martian CO_2 . The figure illustrates most of the important sources and sinks for CO_2 on early Mars. Outgassing from the mantle is considered to be the ultimate source as the atmosphere is secondary, and is not derived directly from the solar nebula⁶. Comparison with the CO_2 and carbonate budgets of the Earth and Venus suggests that Mars could easily derive an atmosphere with a pressure of many bars from this source.

Keeping it is another matter. Weathering of surface rocks and carbonate formation are estimated to cause the atmosphere to collapse on a timescale of 10^7

years according to Pollack *et al.*⁷. These authors invoke volcanic recycling to continually reflux CO_2 back into the atmosphere. Mars was very active volcanically in early times, but as this activity waned, the atmosphere more-or-less irreversibly recombined with the surface. It may have halted only when the surface pressure reached the triple point of water (about 6 millibar), below which water will not stably exist, even transiently, and carbonate formation would cease⁸. Ground-based spectroscopic detection of the carbonate-bearing mineral scapolite in martian dust and soil has been reported recently⁹. (It is interesting to note that without life, Earth's atmosphere would also recombine with surface rocks, possibly leaving our planet in a Mars-like state, with only a thin, cold atmosphere of argon and CO_2 .)

Impacts could have several effects on the early atmosphere (see figure). One is impact erosion. Impactors could also be a source of atmosphere if they are moving slowly enough. This is fine for asteroids, but Melosh states that comets generally move so fast that the vapour plume produced cannot be retained on Mars or the Earth (personal communication). Thus the idea that the Earth owes its oceans to cometary bombardment long ago may have to be abandoned. Impacts on Mars could also exhume and release carbonates. Schultz notes that erosion was much more intense on Mars before the formation of the last great impact basin, Argyre, than after¹⁰.

Perhaps the collapse of the martian atmosphere (recombination with the surface) was more tied to the impact history of Mars than to its volcanic history. Whatever the controlling factors, impact erosion is expected to occur, and may solve some old mysteries. Mars is depleted in argon compared to Earth and Venus, and this has been used to argue that Mars was less efficiently outgassed. Preferential impact erosion of the martian atmosphere may make this unnecessary¹. Mars may be as efficiently outgassed as Earth, and thus may have released to its surface as much or more water per gram as Earth. □

William B. McKinnon is in the Department of Earth and Planetary Sciences and McDonnell Center for the Space Sciences, Washington University, Saint Louis, Missouri 63130, USA.

1. Melosh, H.J. & Vickery, A.M. *Nature* **338**, 487–489 (1989).
2. Cameron, A.G.W. *Icarus* **56**, 195–201 (1983).
3. Walker, J.G.C. *Icarus* **68**, 87–98 (1987).
4. Ahrens, T.J. & O'Keefe, J.D. *Int. J. Impact Engng.* **5**, 13–32 (1987).
5. Stewart, G.R. *Nature* **335**, 496–497 (1989).
6. Prinn, R.G. & Fegley, B. A. *Rev. Earth planet. Sci.* **15**, 171–212 (1987).
7. Pollack, J.B., Kasting, J.F., Richardson, S.M. & Poliakoff, K. *Icarus* **71**, 203–224 (1987).
8. Kahn, R. *Icarus* **62**, 175–190 (1985).
9. Clark, R.N., Swayze, G.A. & Singer, R.B. *Bull. Am. Astron. Soc.* **20**, 849 (1988).
10. Schultz, P.H. in *MECA Workshop on the Evolution of the Martian Atmosphere* 22–23 (Lunar Planetary Inst., Houston, 1985).

Spinning in space

THE traditional space rocket ejects a stream of hot combustion gases. This is almost the only way of generating the huge but short-lived thrust needed to lift a spacecraft away from the Earth's surface. But once in space, a satellite or probe could be just as well manoeuvred by a very small thrust applied for a very long time. One possibility is to exploit solar or nuclear power to accelerate a continuous exhaust stream of electrons and heavy ions. But Daedalus has another suggestion. His new rocket motor doesn't eject any kind of gas or plasma. Instead it fires out a *solid* exhaust.

His ingenious propulsion system melts-spins a glass or polymeric material into a transparent fibre, writes alternate positive and negative charges on it from electron and ion sources, and then accelerates it to high velocity in an electrostatic linear motor. No net charge accumulates on the spacecraft, as the ejected fibre is on average electrically neutral. And in the microgravity and hard vacuum of space, it could be drawn to extremes of small diameter, high tenacity and high final velocity. Daedalus calculates that a 0.5-micrometre fibre ejected from a spacecraft at 4 km per second would generate about 10 millinewtons of continuous thrust from a few watts of power, easily drawn from solar panels. Over the months of a long space mission, this could build up a useful velocity increment, or make possible delicate manoeuvring, for a tolerable loss of mass. And as a final brilliant stroke, Daedalus will use the ejected fibre as an optical communication channel to link the spacecraft back to Earth.

The 'Space Spider' poses some intriguing problems. DREADCO's mathematicians are already wrestling with the gravitational dynamics of the ejected fibre, so as to establish trajectories for the Spider which will not stretch or break its ever-extending fibre. This would certainly break at the Earth end if any attempt were made to lead it down through the atmosphere. It will have to be anchored to a command satellite, orbiting in a plane normal to the Spider's trajectory: this would waggle the fibre but would not stretch or entangle it.

One serious snag is that few optical fibres are usefully transparent over more than 100 km or so. So Daedalus, cunningly, plans to equip his space fibre with distributed optical gain. DREADCO's chemists are devising super-radiant glasses which will lase in the sunlight of space. An optical pulse passing down such a glass fibre will be continuously amplified, and will travel unattenuated for any distance.

The Space Spider will open up whole new strategies of secure-communication deep-space probes. Sadly, only one or two can be flying at any one time, or they will surely get tangled up. David Jones

Regulation of kinase activity

SIR—In a recent News and Views article, Hardie¹ described the emerging evidence that protein kinases can be inhibited by interacting with substrate-like sequences. These sequences are present either within the same molecule, as in kinase C, or in a different molecule, as in the regulatory subunits of cyclic AMP-dependent kinases. I would like to point out that an inhibitory role of amino-terminal sequences over the carboxy-terminal catalytic domain has also been shown for p60^{v-src}, a member of the non-receptor family of tyrosine kinases². The mechanism whereby this negative regulation occurs has not yet been addressed, although studies performed on p60^{c-src}, the cellular (c) homologue of the viral (v) protein, support the idea of substrate-like sequences being involved.

Amino-acid positions 90 and 92 of p60^{c-src} are tyrosine residues, one of which is surrounded by acidic amino acids as are many tyrosine-kinase target sequences (see figure). These sequences are located in the amino-terminal modulatory domain of the molecule within a stretch of about 50 amino acids that are conserved among all the members of the *src* family. This region has been called the SH3 or A box³ and it is not present in the receptor class of tyrosine kinases.

Activated p60^{c-src} molecules, such as those bound to middle-T antigen of polyomavirus, or those present in certain neuroblastoma cell lines, are phosphorylated on tyrosine at their amino termini^{4,5}. Based on their neighbouring sequences, tyrosine residues 90 and 92 represent the most likely targets of phosphorylation within this amino-terminal region. An

interpretation of these results is that in the off state the catalytic carboxy-terminal domain interacts with the amino-terminal substrate-like sequences. Phosphorylation of the amino-terminal site would only occur when the kinase is activated, presumably by an induced conformational change. Phosphorylation at these sites may allow maintenance of the on state.

A similar effect is observed with certain amino-acid substitutions of p60^{c-src}. These are Gly 63→Asp, Arg 95→Trp, and Thr 96→Ile, which are present in the viral protein p60^{v-src}, and are sufficient to increase the specific activity of the kinase⁶. The recombinant chimaeras between v-*src* and c-*src* used in these studies⁷ are shown schematically in the figure. Among these three mutations, the crucial one is likely to be Arg 95→Trp, because Gly 63→Asp when present alone in p60^{c-src} is silent, and Thr 96→Ile is not conserved in other strains of Rous sarcoma virus. Arg 95 is three residues away from the tyrosine-phosphorylation site and its presence could weaken the interaction with the kinase domain, thereby releasing the inhibition and allowing constitutive activation of the kinase.

A more rigorous proof that Arg 95 lies within a kinase regulatory domain has been obtained by Potts *et al.*⁷, who find that the Arg 95→Trp mutation alone is sufficient to activate p60^{c-src}. In addition, this group and also L. Fox, K. Frost and J.S. Brugge (personal communication), have shown that mutations at either tyrosines 90 or 92, or the deletion of amino acids 92–95, activate the transforming potential and the kinase activity of p60^{c-src}. Thus, it is possible that an intramolecular

negative control of kinase activity among the *src* family resides within substrate-like sequences outside the catalytic domain.

CARLA GRANDORI

The Rockefeller University,
Box 102, 1230 York Ave,
New York,
New York 10021, USA

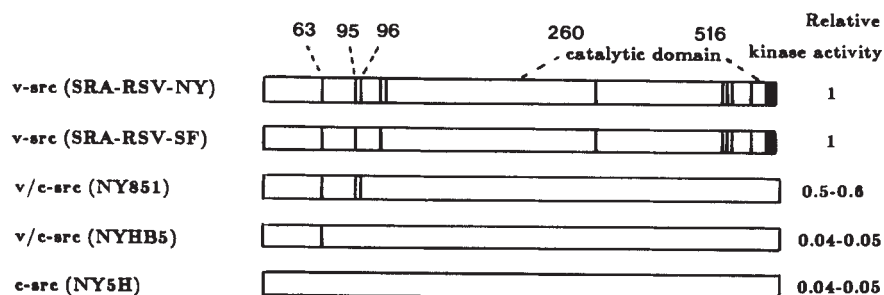
1. Hardie, G. *Nature* **335**, 592–593 (1988).
2. Brugge, J.S. & Darrow, D. *J. biol. Chem.* **259**, 1550–1557 (1981).
3. Pawson, T. *Oncogene* **3**, 491–495 (1988).
4. Yonemoto, W., Jarvis-Morar, M., Brugge, J.S., Bolen, J.B. & Israel, M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4568–4572 (1985).
5. Bolen, J.B., Rosen, N. & Israel, M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 7275–7279 (1985).
6. Kato, J.Y. *et al. Molec. cell. Biol.* **6**, 4155–4160 (1986).
7. Potts, W.M., Reynolds, A.B., Lansing, T.J. & Parsons, T.J. *Oncogene Res.* **3**, 343–355 (1989).

Tale of two serines

SIR—In a recent paper¹, Brenner used the fact that serine is encoded by two non-linked codon types, UCN and AGY, in conjunction with his observation that within several enzyme families catalytic serine residues have different codon representations, to propose that these serines evolved convergently by single substitutions in cysteine or threonine codons (UGY and ACN, respectively), the latter being catalytic residues in ancestral enzymes of each class. This proposal, however, fails to account for the serine codon representations of two groups of proteins.

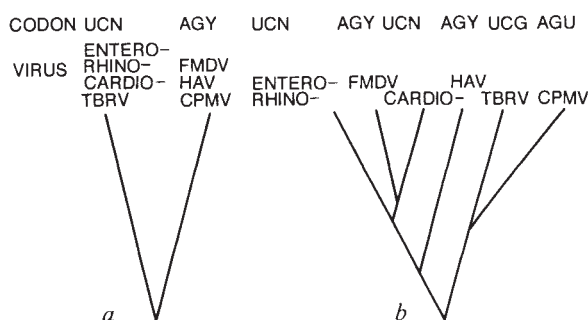
First, the chymotrypsin-like proteases from *Streptomyces griseus*, SGPA and SGPB (ref. 2): in these enzymes, the catalytic serine residues are encoded by AGU and UCC, respectively, but the 61 per cent identity of amino acids between SGPA and SGPB, and their similar sizes, make it highly improbable that they belong to two different phylogenetic lineages, as predicted by Brenner's hypothesis. Second, the viral replicative proteins containing the widespread nucleoside-5'-triphosphate (NTP)-binding motif (refs 3–5) Gly-X-X-X-Gly-Lys-Ser/Thr (GXXXXGK S/T) where X is any amino acid: as shown in the figure and table, the lineages of the GKS-containing proteins of picornavirus, comovirus and nepovirus derived from serine codon types are incompatible with the phylogeny arising from sequence comparisons (compare *a* and *b* in the figure). Moreover, the conspicuous absence in this family of a GKT-containing protein is at odds with Brenner's additional suggestion that, in the case of the NTP-binding motif, threonine might be an evolutionary intermediate between the two kinds of serine, rather than their predecessor. It seems to be the case, in the proteins of this family, that threonine is not acceptable in the NTP-binding motif and that evolutionary transitions between UCN and AGY

W
* * 95
p60^{c-src} (amino acid 81–106) GGVTTTFVALYDYESRTETDLSFKKGE



Amino acids 81 to 106 of p60^{c-src}. W, substitution of Arg→Trp as in p60^{v-src}, asterisks are above tyrosines 90 and 92, underlines, acidic amino acids surrounding the tyrosine-phosphorylation sites. Lower panel, schematic diagram of various *src* proteins from: Schmidt Ruppin subgroup A Rous sarcoma virus, New York strain (SRA-RSV-NY); San Francisco strain (SRA-RSV-SF); chimaeric viral and cellular *src* recombinant viruses (NY851 and NYHB5); a non-mutated cellular *src* recombinant virus (NY5H). Mutations 63, 95 and 96 are indicated; vertical lines show amino-acid substitutions in other regions of the viral protein as compared with the cellular *src* protein. The relative kinase activity was measured by *in vitro* phosphorylation of enolase as previously described⁵.

Phylogeny of putative NTPases of picornavirus, comovirus and nepovirus. *a*, Phylogenetic scheme derived from the data of the table according to Brenner's hypothesis; *b*, phylogenetic tree generated by comparison of amino-acid sequences of evolutionary conserved segments of putative NTPases using a rate-independent distance matrix method^{7,8}. Only the branching order is shown; the branch lengths were chosen arbitrarily. The branching order of this tree is identical to that generated for viral RNA polymerases⁹ and for capsid proteins¹⁰, and presumably reflects the phylogeny of viral genomes as a whole. Codon representations of serine in the GKS consensus are shown. Where, in a group of viruses, only one codon series is utilized, the branching order was not further specified.



codons occur without GKT intermediates.

In two other families of viral GKS/T-containing proteins serine is encoded almost exclusively by UCN, with AGY occurring only once (see table). Again, it is unreasonable to suppose that the potexvirus gene containing AGU originated from a separate line of descent. Both these families, however, include GKT-containing proteins seemingly making the evolution of differentially encoded serines through threonine intermediates a possi-

bility. Nevertheless, it seems that if this occurred then it did so only rarely.

Although our survey of serine codon usage in putative viral NTPases does not support Brenner's hypothesis, it exposes some intriguing variations in the evolutionary mechanisms of different phylogenetic lineages. At least two mechanisms may be invoked to explain how the UCN to AGY transition occurs without loss of serine at the enzyme active site. The first of these requires the simultaneous change of two adjacent bases. Given the high error rate of RNA replication⁶, this mechanism is more feasible for RNA viral genomes than for DNA genomes. An alternative mechanism involves the generation of a new serine codon next to the functionally important one (yielding a

GKSS sequence with the two serines encoded by codons of different series) followed by deletion of the original codon. This mechanism is equally feasible with DNA and RNA genomes and might operate wherever there is no strict constraint on the residue(s) next to the functional serine. Generally, understanding the evolutionary history of serine codons in catalytic centres of each enzyme class requires knowledge of its phylogeny derived from independent data.

EUGENE V. KOONIN

ALEXANDER E. GORBALENYA

*Institute of Poliomyelitis and
Viral Encephalitis,
USSR Academy of Medical Sciences,
142782 Moscow Region,
USSR*

1. Brenner, S. *Nature* **334**, 528–530 (1988).
2. Henderson, G. et al. *J. Bact.* **169**, 3778–3784 (1987).
3. Walker, J.E., Saraste, M.J., Runswick, M.J. & Gay, N.J. *EMBO J.* **1**, 945–951 (1982).
4. Gorbalenya, A.E., Blinov, V.M., Donchenko, A.P. & Koonin, E.V. *J. molec. Evol.* **28** (in the press).
5. Evans, R.K., Haley, B.E. & Roth, D.A. *J. Biol. Chem.* **260**, 7800–7804 (1985).
6. Steinhauer, D.A. & Holland, J.J. *A. Rev. Microbiol.* **41**, 409–433 (1988).
7. Yushmanov, S.V. & Chumakov, K.M. *Molec. Genetika* **3**, 9–15 (1988).
8. Chumakov, K.M. & Yushmanov, S.V. *Molec. biol. Evol.* (in the press).
9. Koonin, E.V., Chumakov, K.M., Yushmanov, S.V. & Gorbalenya, A.E. *Molec. Genetika* **3**, 16–19 (1988).
10. Palmenberg, A.C. in *Molecular Biology of Picornaviruses* (ICN–UCI, in the press).
11. Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P. & Blinov, V.M. *FEBS Lett.* **235** 16–24 (1988).
12. Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P. & Blinov, V.M. (submitted).

Serine codon representations in the nucleotide-binding motif of positive strand RNA viruses

Family of viral 'NTPases'	Consensus sequence	Serine (threonine) codons
Family I		
Alphaviruses	GKS	UCN
Coronavirus	GKS	UCC
Furovirus	GKS	UCN
Hordeiviruses	GKS	UCA
Tobraviruses	GKS	UCG
Potexviruses		
WC1MV p147	GKS	UCU
p26	GKS	UCU
PVX p165	GKS	AGU
p26	GKS	UCC
Tricornaviruses	GKT	ACN
Tobamovirus	GKT	ACC
Tymovirus	GKT	ACA
Family II		
Enteroviruses	GKS	UCN
Rhinoviruses	GKS	UCN
Cardioviruses	GKS	UCN
Aphthoviruses (FMDV)	GKS	AGY
Hepatitis A virus (HAV)	GKS	AGY
Comovirus (CPMV)	GKS	AGU
Nepovirus (TBRV)	GKS	UCG
Family III		
Potyvirus	GKS	UCN
Flaviviruses	GKT	ACN
Pestivirus	GKT	ACA

For sources of sequence data see refs 4, 11 and 12. N, any nucleotide; Y, pyrimidine. The grouping of viral proteins containing the NTP-binding motif is according to refs. 4 and 12. Potexviruses (as well as furoviruses and probably hordeiviruses) have two putative NTPases each⁴. Different species of enterovirus and rhinovirus, and different strains of foot and mouth disease virus (FMDV) and HAV have either C or U in the third position; hence, N or Y is indicated, respectively.

Telomere formation in yeast

SIR—We recently demonstrated that during formation of new telomeres in the yeast *Saccharomyces cerevisiae*, telomeric sequences are often transferred between DNA termini¹. We argued that the most reasonable explanation for this transfer is recombination between DNA termini. In a recent News and Views article², however, Szostak suggested that the telomere resolution reaction³ (the cleavage between two blocks of telomeric sequences that are oriented as a head-to-head inverted repeat^{3,4}) can provide an alternative explanation for our data, a possibility that can be addressed definitively by DNA sequencing. It is not clear to us why this possibility was raised because we stated¹ that our unpublished sequence data support the interpretation presented in the article; that is, the orientation of the transferred repeats is the same as that of the test termini (S.-S. Wang and V.A.Z., in preparation).

Although the sequence data eliminated the resolution model as an explanation for the telomeric transfer, we did not discuss these data specifically in terms of this model¹. The resolution reaction never provided a likely explanation for our results because it requires three events: (1) circularization of linear plasmids bear-

ing telomeric repeats at each end; (2) asymmetrical resolution of the circles thus formed; and (3) telomere formation on an end with the telomeric repeats in the 'wrong'^{1,4} orientation. Not only have none of these processes been demonstrated in yeast, but even symmetrical resolution is inefficient (~1 per cent per cell division)⁴ compared with the sequence transfer we observe.

Because the resolution reaction is excluded unequivocally by DNA sequence data, telomere–telomere recombination remains the only reasonable explanation for the transfer of telomeric sequences that we have observed. Whether or not yeast exploits telomere–telomere recombination in the replication or maintenance of telomeres remains to be determined.

VIRGINIA A. ZAKIAN

ANN F. PLUTA

*Fred Hutchison Cancer Research Center,
1124 Columbia Street,
Seattle,
Washington 98104, USA*

1. Pluta, A.F. & Zakian, V.A. *Nature* **337**, 429–433 (1989).
2. Szostak, J.W. *Nature* **337**, 303–304 (1989).
3. Szostak, J.W. *Cold Spring Harbor Symp. quant. Biol.* **47**, 1187–1194 (1982).
4. Murray, A.W., Claus, T.E. & Szostak, J.W. *Molec. cell. Biol.* **8**, 4642–4650 (1988).

Trials of a lifetime

Alex Comfort

The Retardation of Aging and Disease by Dietary Restriction. By Richard Weindruch and Roy L. Walford. *Charles C. Thomas, Springfield, Illinois: 1989. Pp. 436. \$69.75.*

ONE of the few things we know — and have known for half a century — about the ageing process in mammals is that it can be slowed by caloric restriction. McCay's original finding has been repeatedly challenged and repeatedly re-verified, not only in mammals, the organisms which really preoccupy experimental gerontologists, but also in fish, invertebrates and test organisms generally. The apparent slowing, moreover, is not just a reduction in the diseases of overnutrition. Rather it is a moving to the right of the curve of survival, and the curve seems to move all identifiable landmarks with it: one would confidently predict that pedestrians subject to dietary restriction (DR) would have a peak for road fatalities at a higher age than those who are non-restricted.

Weindruch and Walford have produced a comprehensive book on this entire question. It powerfully concentrates the mind, notably by making it impossible to avoid asking why more effort has not been concentrated on assessing this effect in human beings. The mode of action of DR remains obscure — there are cellular, programme delay, error accumulation, neuroendocrine and immunological hypotheses, and the literature and evidence for all of them is painstakingly reviewed here for the first time. What precisely happens to molecular, physiological and immunological parameters is reviewed in equal detail, a fairly massive undertaking in view of the large range of organisms and the desultory character of much of the research. The purpose of this overview is to prepare us to ask ourselves whether, on the evidence, DR should work in human beings, and, if so, why no concerted attempt is being made to test it.

One can think of a number of answers to the last part of the question — actuarial studies on diet are by nature a two-lifetime project unlikely to attract funding sources wedded to instant gratification; and non-actuarial biomarkers, as the Hiroshima project investigators found, are quite difficult to choose and evaluate. Finding volunteers for lifelong, or even five- to ten-year DR projects might be difficult if significant numbers were needed. The numbers, however, depend on the magnitude of the effect: if four out of six dedicated dieters, evading Makeham's coefficient, lived to >105–106 years of age, people might begin to take notice, though by that time other approaches to ageing control, by genetic manipulation, might have devalued DR as a clinical approach.

One can go on multiplying difficulties, and this, in fact, is what gerontologists have done so far. One must also suspect another and psychological factor, the fear of being thought overzealous in the style of the early rejuvenators. Weindruch and Walford, whose own research credentials are impeccable, confront the timid with reasoned argument and present here a research prospectus which is very difficult to fault or ignore. If we do not wilfully ignore it, we ought to be planning how to put it into effect rather than waste another 20 or so years arguing about it — landmark-

shifting has implications in developing a medicine of rate determination which goes considerably beyond the vulgar pursuit of mere longevity.

It is refreshing to come across a text that is not only readable and a comprehensive review of its subject, but also a challenge to researchers and funding bodies; we can either extend our control over the life cycle or prove the authors wrong by trial. Walford is a respected figure and the founder of gerontoimmunology who is known to carry out dietary trials on himself. Anyone who views that undertaking as eccentric should feel obliged to respond to the admirably presented case contained in this book. □

Alex Comfort, *The Windmill House, The Hill, Cranbrook, Kent TN7 3AH, UK, is Adjunct Professor at the Neuropsychiatric Institute, University of California at Los Angeles, and a former Head of the MRC Research Group on Ageing at University College London.*

Down to Earth

Ian D. R. Mackinnon

Meteorites and the Early Solar System. Edited by John F. Kerridge and Mildred Shapley Matthews. *University of Arizona Press: 1988. Pp. 1,269. \$55, £42.95.*

AT FIRST glance, the study of meteorites must appear to be an unusually obscure discipline, made more so by jargon and a confusion of terms. There have been successful introductory-level, undergraduate texts on the subject, but these have wisely glossed over the debates on interpretation of the meteoritic record. The detail required to do justice to such issues would hardly excite any publisher, and an aspiring author would risk losing tenure at the most tolerant of institutions. It has taken a unique combination to break the mould: an outstanding specialist publisher, and the patience and tenacity of two well-known members of the space-science community. Several excellent books, co-edited by Mildred Matthews, have appeared in the University of Arizona Press "Space Science" series; this most recent publication, co-edited with John Kerridge, maintains the standard.

In 1,200 pages, the editors and 68 collaborating authors make a stellar effort at providing a précis of early Solar System history interpreted through the meteoritic record. The broad sweep of meteorite studies, which cover many scientific disciplines and employ a multitude of techniques, is clearly evident in the tone and content of the volume. Moreover, it's not all petrography and isotopic signatures. Included are well-written summaries of current astrophysical models of pre-nebula and solar nebula evolution, as well as of

models of nebula accretion processes. In many of the contributions attempts have been made to relate models to the observational record and to present the pros and cons of contentious issues. Some of them, such as that linking asteroids and meteorites, are successful; others are less so, but as much because of gaps in the record than any lack of effort.

Although some may quibble with the precise positioning of chapters, or even with the emphasis on Solar System/nebula processes, there is no denying the success of the overall format. Telling questions to be asked of multi-authored volumes are how well concepts are related between different chapters, and whether authors who might not ordinarily be seen sipping tea together at the annual meeting of the minds have been persuaded to collaborate. Here it appears that contributors did bother to read drafts of other chapters, and have delivered the goods. In those chapters where unlikely collaborations have been imposed, good summaries of research have resulted and some have provided new insights which perhaps have surprised even the authors.

The book was in gestation for well over two years, and that time has been fruitfully used. Here we have a coherent and detailed overview of the dominant processes affecting chondrite meteorites, from early nebula formation (or survival?) of precursor grains, to their accretion and incorporation in an asteroidal parent body, followed by various impact processes, ejection from dominantly asteroidal orbits, and then eventual collection and transfer to the laboratory for study. Most chapters specifically address chondrite meteorites only, as these are believed to have undergone the least degree of modification since their formation about 4.5×10^9 yr ago. However,

helpful explanatory references to achondrites such as SNCs (purportedly derived from Mars) and ureilites are scattered throughout. One chapter on igneous activity, which at first blush would be a questionable entry, has been neatly integrated into the rest of the text. The relationship between the terrestrial planets and asteroids, as revealed by meteorite studies, is also well developed in a series of chapters under the heading "Chemistry of Chondrites".

A firm editorial hand is also evident in the highly consistent use of symbols, acronyms, abbreviations and units of measure. In addition, the glossary and useful compilations of fundamental data in five appendices will be appreciated by many readers. The index has received the same thorough treatment — entries under "Meteorites, individual" occupy almost two pages and give a quick synopsis of the chondrites whose characteristics have primarily shaped current thinking on processes of the early Solar System.

In early correspondence on the proposed book, Kerridge made two important conditions for the final product. First, he felt that it needed to be well balanced and cohesive. Second, that it was not to be a vehicle for perpetuating dogma; many meteoriticists need reminding of this point, as it is often all too easy to follow the gurus. No doubt because of that prompting, many authors have made genuine efforts to sketch the nub of controversies where they exist and, more importantly, to suggest means for their eventual resolution. The final chapter on future directions is a thoughtful assessment of meteorite studies and a rich source of ideas for research proposals in this, and related, fields.

Meteoritics has come a long way since the days of Harvey Nininger and Harold Urey, to whose memory this volume is dedicated. Both were in their sunset years during the halcyon days of the Apollo programme, which gave new life to the study of meteorites as well as to that of the Moon. Both played a large part in the development of meteorite research and they would, no doubt, be proud of this singular community achievement. □

Ian D. R. Mackinnon is Director of the Electron Microscope Centre, University of Queensland, St Lucia, Brisbane, Australia 4067.

• *Nature's* next review supplement, Spring Books, appears in the issue of 20 April. The books covered include *Digging into the Past*, Edwin Colbert's autobiography, *AIDS: The HIV Myth* by Jad Adams and *AIDS and Its Metaphors* by Susan Sontag, *Journey into Light*, a biography of C. V. Raman, *Does God Play Dice?* by Ian Stewart, Stuart Sutherland's dictionary of psychology, and two controversial works in archaeology. Among the reviewers are Jeremy Sabloff, Daniel S. Greenberg, Walter Gratzner, Michael Berry, Roy Porter and Robert M. May.

Learning of the Lucky Country

Robert A. Stafford

Nature in Its Greatest Extent: Western Science in the Pacific. Edited by Roy MacLeod and Philip F. Rehbock. *University of Hawaii Press: 1988. Pp.288. £27.20, \$34.*

The Commonwealth of Science: ANZAAS and the Scientific Enterprise in Australasia, 1888–1988. Edited by Roy MacLeod. *Oxford University Press: 1988. Pp.417. £25, \$45.*

THE motive proffered by Defoe's Captain Singleton for his peregrinations — "knowledge of things begat a desire of increasing it" — may serve as a simple explanation for the sustained European effort to penetrate, understand and organize the lands washed by the Pacific Ocean. Because Singleton was modelled on the circumnavigator Dampier, the analogy is particularly apposite, for Dampier's observations of natural phenomena created the vogue for South Seas exploration which endured throughout the eighteenth century and resulted in Britain's colonization of Australia.

With interest now turning to the Pacific basin as the theatre for the next major phase of economic development, it is appropriate that the historical role of Western science in the region should be assessed. Australia having celebrated its bicentenary, it is equally fitting that analysis of the place of science in the culture and economy of the island continent should be included in this analysis. The contributors to these two new books, which complement R. W. Home's *Australian Science in the Making* (Cambridge University Press, 1988), explore some of the more prominent features of what remains in many respects an intellectual *terra incognita*.

The function of the historian as pioneer is especially apparent in *Nature in Its Greatest Extent*. In conjunction with Philip Rehbock, Roy MacLeod has collected ten essays documenting European scientific activity in the Pacific from the eighteenth century to the present. The book opens many doors, revealing the wealth of materials that will enable future researchers to tell us in greater detail how Europe's discovery and exploitation of the lands of the Pacific transformed not only the region's peoples and habitats, but the mental landscape of Europeans. Individual contributions to the volume, however, are variable in quality, while the book as a whole is marred by incoherence of theme. The editors might have supplied a connecting overview in their introduction, but instead they pose questions

which the text fails to answer adequately.

The problem lies in defining the changing nature of the symbiotic relationship which has obtained between Western science and expansionism. Europeans were activated by greed as well as curiosity in coming to the Pacific. Science received patronage during the eras of exploration and settlement in exchange for the promise to deliver new resources for exploitation, new means of subduing hostile environments, effective methods for maintaining European ascendancy and cultural prestige for the metropolitan powers.

None of the essays covering the centuries before the Cold War sufficiently emphasizes that many European scientists were active agents of imperialism rather than mere fact-gatherers engaged in pushing back the frontier of the unknown. By means of their reports, maps, collections, taxonomic classifications and — more directly — their administrative and military duties, scientists facilitated the gradual imposition of European domination over the Pacific. As mediators of this new world, scientists played a large part in moulding European attitudes towards it. Their research agendas and results were, in turn, conditioned by European cultural norms, so that apparent objectivity often masked or reflected subjective metropolitan aspirations and anxieties. The same process was occurring in other regions which, as the editors fail to mention, presented challenges to Western intellectual assumptions and physical capabilities as daunting as those of the Pacific.

The book is divided into three sections. The first concentrates on the scientific content of the voyages of discovery and the tension between science and politics in their planning and execution. The second section, which offers more insightful analysis, examines how European science transplanted to the Australasian colonies sought to fulfil duties assigned by metropolitan savants while developing the impetus to assert its own authority and priorities. The long march of colonial scientists seeking intellectual independence was inexorably intertwined with the movement for political independence.

These essays likewise illustrate that science at the colonial periphery suffered severe constraints in comparison with that practised in Europe. Researchers were few on the ground, facilities rudimentary and funds scarce. Colonial culture often exhibited a philistine attitude towards the scientific enterprise, and the tyranny of distance between Port Moresby and Sydney was as real as that separating the South Pacific from London or Paris.

The final section moves into the twentieth century, when the winds of change which swept away the old empires cleared the ground for the new imperialism of the superpowers and the transnational corpo-

rations. In the interwar years, we learn, a burst of internationalism produced the Pacific Science Association, whose congresses have fostered an enduring tradition of cooperation among Pacific scientists. But following the Second World War, as many colonies discovered that political independence amounted to the perpetuation of subservience as client states of the United States or the Soviet Union, Pacific science traded old masters for new. The stars and stripes and the hammer and sickle now fly over far more scientific vessels or research stations than do the flags of other Pacific nations, or even the stubborn Tricolour.

As the last two chapters demonstrate, American and Soviet science remain as motivated by commercial and strategic concerns as were the scientific establishments which served the European powers during the exploration and colonization of the Pacific. But although the sciences have been employed for centuries as instruments of policy, scientists have with equal consistency shown themselves to be adept at exploiting governments to support research programmes that are ultimately driven by personal ambitions as well as disciplinary goals. It is in explicating the fine detail of this interplay that one of the most exciting frontiers of the history of science in its wider social dimension lies.

In *The Commonwealth of Science*, MacLeod embarks alone as editor to chart the history of science as it has developed in Australia during the twentieth century. On surer ground here than among the long Pacific swells, he builds upon the corpus of research published by the prolific Antipodean community of historians of science, as well as upon his earlier work on the British Association (the model for ANZAAS) and science policy throughout the empire. The story of ANZAAS, the Australian and New Zealand Association for the Advancement of Science, provides the prism through which we are invited to view science in the Antipodes from 1888 to the present. Coinciding with Australia's bicentenary, the ANZAAS centenary represents a convenient benchmark for surveying the evolving style and role of organized natural knowledge in the economic and cultural development of the nation.

The book contains several provocative essays, but again the quality is uneven. In two chapters discussing the foundation and early years of ANZAAS, MacLeod sets a standard of excellence — indeed, of eloquence — which some of his contributors fail to sustain. Institutional histories chronicling various disciplines alternate with analytical chapters which examine the meaning of science, the Australian national identity and man's place in nature. Rather than being a history of ANZAAS, the book provides an overview of the social role of science in Aus-



"The Pathfinder" — Science forging her way into the light (*Daily Telegraph*, 1911).

tralian history. This weakness is its strength, and in scope and penetration *The Commonwealth of Science* has a great deal to recommend it. We might wish, however, that MacLeod himself had completed the chronological narrative in the interests of keeping ANZAAS at the centre of things.

Nevertheless, we are left with an important contribution to the history of science in a former European colony and to the sociological literature on what it means to be Australian. Two recurring themes in the 15 chapters are the tensions between Antipodean colony and European metropolis, and between an imported culture and the alien environment upon which it has been imposed. Science figures squarely in these issues, for it has provided a venue for the articulation of Australian aspirations towards independence while simultaneously serving as a mediating device for the exploitation and understanding of a continent new to the European consciousness. Australia was born to the scientific age, so it is not surprising to learn that ANZAAS has functioned as a central reference point in the continuing adventure of creating an outpost of Western civilization in the South Pacific. It has proven a valuable public resource in a culture often branded as materialistic and anti-intellectual.

It remains to be seen whether Australia

will succeed in forging an effective national science policy. Such a strategy must harness the sciences to the goal of transforming Australia from the status of a supplier of primary products to the distant centres of Western power to that of a truly sovereign nation whose industrial and service sectors have attained critical mass and whose society has made the commitment to education and research-led change which is necessary for self-sustaining growth. As several contributors warn, the odds are at least even that Australians may fail, and that, as the Lucky Country devolves into a banana republic, the nation's tradition of scientific excellence will be dissipated like so many of her natural resources.

There are healthy signs, however, that this grim prognosis can and will be avoided by policy makers, and that Australia will maintain its reputation as the land of the second chance. The upsurge of interest in the role of science in the nation's history is one such encouraging sign, and these two books represent the cutting edge of the reappraisal. Anyone interested in the fate of Australian science, or indeed in the future of the country, should read *The Commonwealth of Science*. □

Robert A. Stafford is in the Department of History, La Trobe University, Bundoora, Victoria, Australia 3083.

Divisions in botany

Deborah Charlesworth

Mutation, Developmental Selection, and Plant Evolution. By Edward J. Klekowski, Jr. Columbia University Press: 1988. Pp.373. \$55.

EDWARD Klekowski has made a brave attempt to put together all the available information about mutation in higher plants. His aim is to use the data to support the argument that genetic loads in plants are increased, in comparison to those in animals, by somatic mutation.

The germ cells of higher plants are differentiated anew each reproductive season from several separate meristems, rather than being sequestered in a permanent germ line as in multicellular animals. This means that the number of mitotic cell divisions between the zygote stage and meiosis in the germ cells, which in a plant are formed by a line of cell descent, may on average be much higher than in, say, *Drosophila* or a female mammal (although the number is, of course, higher in male mammals). If there is some probability of a somatic mutation arising at each cell division, the frequency of mutations among the gametes produced by a tree increases with the number of mitoses, which in plants is correlated with age.

Evidence for a correlation between the number of cell divisions before meiosis and rate of mutation has recently been provided by Miyata's analysis of the rates of evolution of sex-linked loci in mammals, which have many more divisions in the male germ line than in the female (*Cold Spr. Harb. Symp. Quant. Biol.* **LII**, 863–867; 1987). Thus the mutation rate per generation should be higher in a long-lived plant, such as a tree, than in shorter-lived species. An old tree, or an old clone of a fern species, would also be expected to be chimaeric, with different meristems of the same genetic individual carrying different and independently generated mutations. In the book, a mass of anatomical and developmental detail on meristems illustrates the various patterns that might occur.

Klekowski devotes most of his text to evidence for these ideas. Unfortunately, it is difficult to estimate mutation rates and to compare species with different life spans, and the few data on mutation in plants are mostly from short-lived species. Because a species with characteristics that tend to increase the mutation rate would probably experience selection to reduce it, such estimates might in any case not show the expected differences. Klekowski therefore discusses mutational processes

and error correction mechanisms in great detail, but as so little is known about these in plants it is difficult to make the necessary comparisons. The frequency of mutant gametes in plants of different age, and the existence of chimaeras, could also be tested, but here again the data are too limited to be of much use.

In the absence of direct tests of the hypothesis, Klekowski marshalls many pieces of circumstantial evidence that agree with his expectations. This necessarily yields a somewhat confusing picture, as such arguments can be wrong in at least two ways. First, some of the phenomena may not be due to genetic load; for example, although genetic load could be the cause of low numbers of seeds per

ovule in trees, other explanations such as selection for high pollen production in these plants, are also possible. Second, interpretations other than Klekowski's could explain differences between the genetic loads of populations. Differences in breeding systems, for example, are largely ignored in the book.

Klekowski therefore does not make a watertight argument for his hypothesis. But, although his book is hard to read because of the diversity of the information presented (a glossary would have helped), it is certainly interesting and rich in ideas for future research. □

Deborah Charlesworth is in the Department of Ecology and Evolution, University of Chicago, Chicago, Illinois 60637, USA.

Vorsprung durch biotechnologie

Stephen Oliver

Biotechnology Focus 1: Fundamentals, Applications, Information. Edited by R.K. Finn and P. Prave. Hanser, Munich/Oxford University Press, New York: 1988. Pp.436. DM128, \$85.

RIGHTLY or wrongly, English has become the international language of science. This is not to say that there is no need for scientific works to be written in other languages, but it does mean that translations into English must be viewed critically.

The American editor of *Biotechnology Focus* justifies the book's translation from German by asserting that in Europe, unlike the United States, genetic engineering has not excluded microbiology and chemical engineering from the area of biotechnology. I am sure there are those at, say, Madison or the Massachusetts Institute of Technology who would take issue with him. The other contention is that the book will unlock a literature unavailable to English-reading scientists. In fact, the principal biotechnologists in the German-speaking world habitually publish in English and less than 12 per cent of the references in *Biotechnology Focus* are to works in German. The longest chapter is on fungal pathogenicity, which is not an obviously biotechnological subject. This chapter is also one of those with the most German-language works in its bibliography; but it does not provide a ready entry to the German literature because only one of the 61 works (in all languages) quoted is actually referred to in the text. Clearly, editorial rigour has failed on one side of the Atlantic or the other, perhaps a case of passing the buck or *Verantwortung abschieben*.

Biotechnology Focus is described as a yearbook, but it is not in the mould of the

American Society of Microbiology's prestigious "Microbiology" series. Most of the contributions are of broad scope, such as the excellent chapter on scale-up theory by Hempel. This, however, sometimes makes them rather too general, as is the case with the contribution on computer control of antibiotic production. Sometimes a very general title disguises a very specific article — "Fundamental Methods of Genetics" mainly deals with the details of protoplast fusion technology using the hydrocarbon-metabolizing yeast, *Yarrowia lipolytica*. A great deal of experimental detail can be found in the book, for example in the account of cell immobilization. But this chapter appears in a section labelled "Applied Biotechnology", and so it is curious that the protocols described are for bench-scale work only. Overall, one has the impression that the editors did not have a clear idea of the book's purpose and so the authors have struggled manfully with an inadequate brief.

The topicality that might be expected of a yearbook is provided by an "Information" section. This is a good idea which is spoilt by the time-lag involved in translation — a company profile has Biogen still operating in Geneva, and the market values of bulk bioproducts are given in 1982 deutschmarks. The German-speaking world has made, and is making, an outstanding contribution to biotechnology; it is sad to see it so poorly represented. □

Stephen Oliver is a Professor in the Department of Biochemistry and Applied Molecular Biology, and Director of the Manchester Biotechnology Centre, UMIST, PO Box 88, Manchester M60 1QD, UK.

● Two books published late last year will be useful to researchers in biotechnology who have an eye on the potential commercial value of their work. *Protecting Biotechnology Inventions: A Guide for Scientists* is by Roman Saliwanchik and is published by Science Tech Publishers, Madison, Wisconsin (distributed outside North America by Springer-Verlag); *Patents: A Basic Guide to Patenting in Biotechnology* is by R. S. Crespi and is published by Cambridge University Press.

Helium isotope ratios in circum-Pacific volcanic arcs

R. Poreda* & H. Craig

Isotope Laboratory, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

* Present address: Department of Geological Sciences, University of Rochester, Rochester, New York 14627, USA

Volcanoes in the 'Ring of Fire' surrounding the Pacific Ocean are sited on tectonic arc segments marking the great subduction zones where oceanic crust returns to the mantle. Helium isotope ratios in volcanic gases along these arcs are close to those found in mid-ocean-ridge basalts, revealing the presence of primordial ^3He released from the wedge of mantle material above the sinking plate. These results show that although the subduction of oceanic crust drives the arc volcanism, the subducted crust itself does not contribute a major fraction of the upwelling magma.

LAVAS associated with subduction, broadly termed arc volcanics, range in composition from basalt to dacite. Although a number of source regions and models have been suggested for the origin of arc magmas¹⁻⁵, a unified model has been difficult

to construct because the isotopic and petrological signatures often require different source contributions. Craig *et al.*⁶, who measured $^3\text{He}/^4\text{He}$ ratios in volcanic gases from Japan, the Marianas and Mt Lassen, showed that the dominant source of helium was the mantle wedge rather than subducted oceanic crust or sediment, both of which are rich in radiogenic ^4He . We expand on that initial work to assess $^3\text{He}/^4\text{He}$ ratios in all the North Pacific volcanic arc systems with respect to the role of the mantle wedge and the nature of the subducted crust in oceanic and continental arcs. Our most detailed study was in the Aleutian-Alaskan arc, a major feature erupting both tholeiitic and calc-alkaline lavas and grading from an oceanic arc on the west to a continental terrain in the east.

Helium isotope measurements were made on volcanic gases associated with recent arc volcanism. We sampled summit fumaroles whenever possible to minimize interaction of the gases with older crust enriched in radiogenic ^4He . Helium in phenocrysts from recent subaerial or submarine lavas was analysed by crushing in vacuum⁷. Figure 1 shows the $^3\text{He}/^4\text{He}$ ratios in the nine volcanic arc terrains studied: Table 1 lists these and other data for the individual volcanoes.

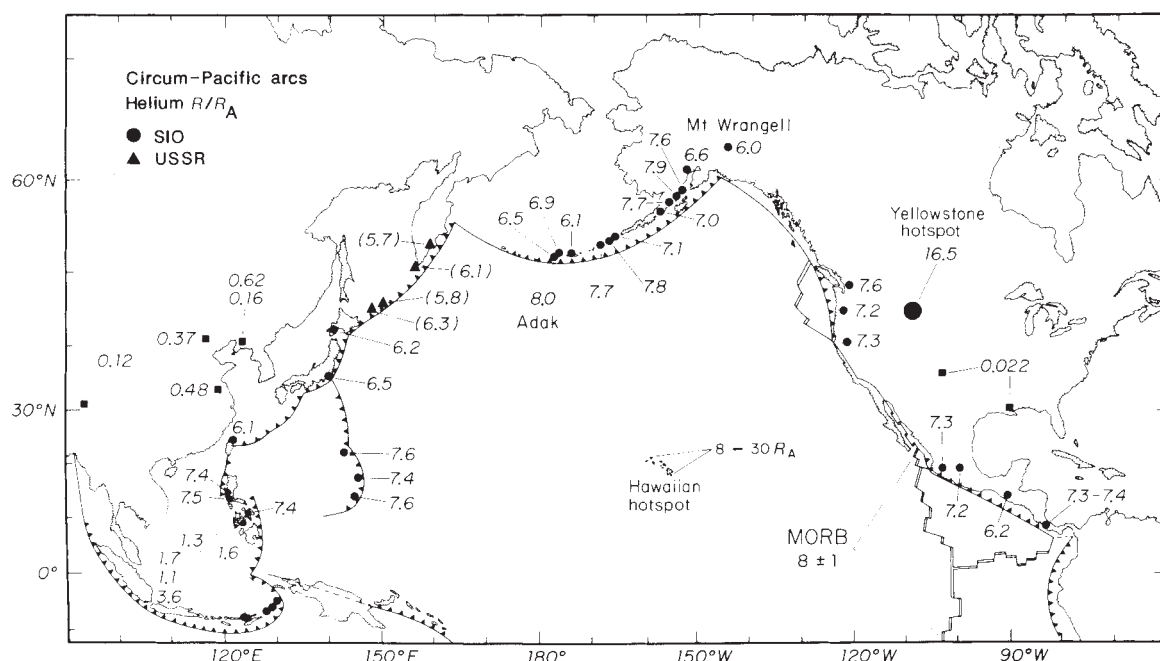


FIG. 1 Helium isotope ratios (relative to atmospheric He) in circum-Pacific volcanic gases and lavas, from this work (circles) and ref. 17 (triangles). A few data for the continental crust of China and the United States (squares) are shown for comparison, as are values for the Yellowstone and Hawaiian hotspots and for MORBs on the East Pacific Rise. Because radiogenic He has almost no ^3He ($R/R_A \approx 0.02$) the data show that the dominant source of helium in volcanic arcs is mantle (MORB) He with $R/R_A = 8$. Most arcs have He ratios from 7 to 8 R_A , with a general mean of $\sim 7.5 R_A$, corresponding to X_M , the fraction of He derived from the mantle, $\sim 7.5/8$, that is, 94%. The Kurile-Honshu-Ryukyu arc system, including Japan, Taiwan, Kamchatka and the Kuriles, has a somewhat greater contribution of crustal or radiogenic

He, with a mean $R \approx 6.4 R_A$ and $X_M \approx 80\%$. Similar values are found at Mts Spurr and Wrangell, volcanoes of the Aleutian-Alaskan arc system that are built on continental crust. The Banda arc, in the eastern sector of the Indonesian system, is caused by continental cratonic material (Australian plate) underthrusting oceanic crust. In this arc there is a much greater dilution of MORB He by the crustal component from the downgoing plate: R/R_A grades from 3.6 to ~ 1.4 , proceeding eastward from the junction with the oceanic Sunda arc at Timor to the Banda Islands. The absolute flux of ^3He from the mantle is known approximately²⁵, so that volcanic arc fluxes of other volatiles can be derived from their ratios to ^3He in arc lavas and gases.

The Aleutian–Alaskan arc

Olivine phenocrysts from Mt Adagdak on Adak, and summit fumaroles on five volcanoes, Mts Okmok, Makushin, Griggs, Douglas and Augustine, have $^3\text{He}/^4\text{He}$ ratios of $R/R_A = 7.6\text{--}8.0$ (where R_A is the $^3\text{He}/^4\text{He}$ ratio in air), the highest volcanic arc values we have observed. These ratios are within the range for mid-ocean-ridge basalts ($R/R_A = 8 \pm 1$; see, for example, refs 8 and 9) which agrees with the conclusion reached previously⁶ that the mantle is the dominant source of helium in arc fluids. Along the entire length of this arc system from the Aleutian islands of Adak and Umnak, built on oceanic crust, to the Alaskan volcanoes Mt Douglas and Mt Augustine built on a

floor of Mesozoic sediments, there is no indication of any alteration in the $^3\text{He}/^4\text{He}$ ratios from input of radiogenic ^4He , even though trace-element and radiogenic isotope data show that sediments have contributed to these arc lavas^{4,10,11}. At the eastern extremity of the arc, however, where the continental crust thickens and the trench–volcano gap abruptly widens¹², the lower $^3\text{He}/^4\text{He}$ ratios at Mts Spurr and Wrangell (6.6 and $6.0 R_A$) reflect significant contributions ($\sim 20\%$) of crustal He from subducting terrigenous sediments¹² or the overriding continental plate.

Helium in volcanic gases consists of mantle He, radiogenic or 'crustal' He ($R/R_A < 0.02$), and atmospheric He. The atmos-

TABLE 1 Helium isotope ratios in circum-Pacific volcanic arc samples

Sample number	Location	Sample type*	R/R_A	He/Ne (He/Ne_A)	R_C/R_A †	T (°C)	$\text{He}/\text{NC}\ddagger$ (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	Ref.
ALEUTIAN ISLANDS									
Adak Island									
AL-25-81	North coast	HS	6.09	11.3	6.58	24.0	51.7		
AL-30-81	Mt Adagdak	OL	7.94	100	8.01	[He] = $9.0 \times 10^{-9} \text{ cm}^3 \text{ g}^{-1}$		0.7031 ± 3	32
Great Sitkin Island									
AL-26-81	Big Fox Creek	FF	3.95	2.2	6.49	97.0	14.0		
AL-27-81	Big Fox Creek	FF	6.86	193	6.89	97.6	—		
Atka Island: Kliuchef Volcano									
AL-34-81	Milky River	FF	6.00	30.2	6.15	96.0	36.0	0.7033 ± 1	32, 41
AL-35-81	Fum. field (615 m)	AS	5.22	17.4	5.40	93.0	38.0		
AL-37-81	Site A (410 m)	AS	5.06	34.8	5.17	97.0	59.0		
AL-38-81	Site B (410 m)	MP	5.20	61.0	5.26	84.0	82.3		
Umnak Island									
AL-17-81	Okmok volcano	GS	7.67	75	7.75	7.0	128	0.7032 ± 2	10, 32
AL-20-81	Geyser bight	HS	7.41	70	7.50	81.0	105		
Unalaska Island: Makushin volcano									
RM-82-MS	Summit	SF	7.83	1,500	7.83	96.0	250	0.7031 ± 2	10, 32
RM-80-MV2	Makushin valley	FF	6.60	110	6.65	—	134		
RM-83-57	Field No. 7	FF	5.91	300	5.93	—	131		
RM-82-WF	West flank	FF	5.04	70	5.10	—	78		
RM-80-MV1	Makushin valley	FF	4.91	37	5.01	98.0	66		
AL-3-81	Makushin valley	FF	4.97	94	5.01	97.2	105		
AL-6-81	Glacier valley	FF	4.52	53	4.58	97.0	58		
RM-82-GV1	Glacier valley	FF	4.04	11.4	4.30	152	53		
AL-5-81	Glacier valley	AS	3.80	24	3.91	92.0	40		
RM-83-77	Test well	W	3.68	41	3.74	193	306		
RM-83-41	Chloride spring	HS	1.27	1.5	1.60	—	3.1		
Akutan Island									
AL-9-81	East flank	AS	7.06	124	7.10	86.6	157	0.7032 ± 2	10, 32
AL-11-81	Hot Spring bay	HS	5.23	4.8	6.10	84.5	14.9		
ALASKAN PENINSULA									
AL-24-81	Gas rocks	S	6.91	114	6.95	26.0	6,140		
K-78-G9	Mt Griggs	SF	7.49	34.8	7.66	96.0	—		
RM-82-DG	Mt Douglas	SF	7.89	850	7.89	93.5	—		
78-A-80G	Augustine volcano	SF	5.90	3.8	7.66	97.2	38.3		
RM-82-AG4	Augustine volcano	SF	7.55	135	7.60	120	—		
RM-82-SPR	Mt Spurr	SF	6.40	24.0	6.62	94.1	—	$0.7037 \pm 3\%$	
RM-82-WR	Mt Wrangell	SF	5.30	6.1	6.05	—	—	$0.7035 \pm 3\%$	
AL-113	Mt Wrangell	SF	6.02	400	6.03	150	1,790		
CASCADES									
	Mt Baker	SF	7.61	900	7.62	—	3,416		
	Mt Hood	SF	7.17	112	7.20	—	2,015	0.7035 ± 3	42, 43
L-2(SW)	Lassen park	AS	7.29	3,000	7.29	87	710	0.7036 ± 3	42, 43
L-8(BH)	Lassen park	AS	7.06	520	7.08	90	—		

The $^3\text{He}/^4\text{He}$ measurements were made on a Clarke-type double-collecting mass spectrometer (GAD) as described previously⁷⁻⁹. In all cases helium was separated from neon on activated charcoal at 37 K to eliminate effects of variable He/Ne ratios on the measured $^3\text{He}/^4\text{He}$ ratios of samples and standards. A secondary standard (Yellowstone Park gas: $R/R_A = 16.45$, $\text{He}/\text{Ne} > 1,000$) was used. Neon was also measured and the He/Ne ratios (column 5) were used to correct the measured $^3\text{He}/^4\text{He}$ ratios, assuming all Ne to be atmospheric. Precision of the $^3\text{He}/^4\text{He}$ ratios is 1%, unless otherwise stated. Total gas chemistry and ^{13}C data will be reported elsewhere.

* Sample key: SF, Summit fumarole; FF, flank fumarole; AS, acid spring; MP, mudpot; HS, hot spring; GS, gas seep; W, well; OL, olivine; CPX, pyroxene; F, fumarole. Data for OL and CPX examples include, at column 7, the He concentration in the phenocrysts, in $10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$.

† R_C is the $^3\text{He}/^4\text{He}$ ratio corrected for atmospheric He contamination¹⁰: $R_C = (RN - R_A N_A)/(N - N_A)$, where R is the $^3\text{He}/^4\text{He}$ ratio, $N = \text{He}/\text{Ne}$, and A denotes the 'atmospheric' ratios. N_A is taken as the He/Ne ratio in air (0.288) for most fumaroles, or in water solution at 10 °C (0.230) for most hot-spring samples.

pheric component (obtained from the He/Ne ratio⁶) includes both entrained air in the natural feature and air added as a sampling artefact. The extensive data on Makushin volcano fumaroles show that the atmospheric and radiogenic components are correlated (see Table 1), indicating that the atmospheric component has for the most part been added in the crustal environment which contributed the radiogenic component.

Western North America

Three volcanoes of the Cascades have $^3\text{He}/^4\text{He}$ ratios of 7.2–7.6 R_A in summit fumaroles and acid springs. Mts Baker, Hood and Lassen represent the northern, central and southern points

of the High Cascades respectively, but whereas the first two are andesitic volcanoes, Lassen is a dacite dome. Thus there is no evidence for a correlation of $^3\text{He}/^4\text{He}$ ratios with the degree of magmatic differentiation in these volcanoes.

In the Trans-Mexican volcanic belt, two high-temperature geothermal systems, Los Azufres and La Primavera, have similar ratios, $R = 7.3 R_A$. Following the Middle American arc into Guatemala, the Zunil field associated with Santiaguito volcano¹³ has fumaroles with distinctly lower values of $R = 6.2 R_A$ that are boiling off from a deep chloride-rich water resident in older country rock, so that the $^3\text{He}/^4\text{He}$ ratio is probably a lower limit. In Costa Rica, a summit fumarole on Poas volcano and

TABLE 1 (continued) Helium isotope ratios in circum-Pacific volcanic arc samples

Sample number	Location	Sample type*	R/R_A	$\frac{\text{He}/\text{Ne}}{(\text{He}/\text{Ne})_A}$	R_C/R_A †	T (°C)	He/NC‡ (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	Ref.
MEXICO									
	La Primavera	W	7.34	2,500	7.34	—	1,636	0.7039 ± 3	44
	La Primavera	F	7.16	55	7.26	95.0	184		
	Los Azufres	W	7.21	—	—	—	—		
GUATEMALA									
GM-1-80	Zunil field	AS	5.95	21.1	6.18	88.0	—	0.7039 ± 1	45, 46
GM-3-80	Zunil field	AS	6.01	—	—	25.0	259		
GM-8-80	Zunil field	W	4.19	>100	4.22	96.0	17.9		
	Pacaya volcano	OL	6.8 ± 1.5	—	—	$[\text{He}] = 4.8 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$		0.7039	45, 46
COSTA RICA									
	Poas volcano	SF	6.84	14.4	7.20	315	145	0.7038 ± 1	47
85-3130	Marivalles	W	7.20	36	7.38	—	177		
85-3131	Marivalles	W	6.43	90	6.49	—	337		
85-3132	Marivalles	W	7.33	160	7.37	—	725		
85-3136	Marivalles	W	6.97	72	7.05	—	390		
MARIANAS									
AG-1	Agrigan volcano	HS	6.92	10.6	7.42	46.0	—	0.7036	0.7035 ± 1
TT192D30 ¶	Eifuku seamount	OL	6.66	—	7.65	$[\text{He}] = 1.4 \times 10^{-9} \text{ cm}^3 \text{ g}^{-1}$			
TT192D01 ¶	Ruby seamount	OL	7.06	—	7.62	$[\text{He}] = 3.4 \times 10^{-9} \text{ cm}^3 \text{ g}^{-1}$			
	Ruby seamount	CPX	6.90	—	7.22	$[\text{He}] = 6.4 \times 10^{-9} \text{ cm}^3 \text{ g}^{-1}$			
PHILIPPINES									
PHIL-13	Mt Pinatubo	SF	7.43	500	7.44	97.5	402	0.7044	16
PHIL-19	Biliran Island	SF	7.41	960	7.42	98.0	205	0.7048	16
PHIL-21	Taal volcano	SF	7.45	100	7.50	99.0	476		
TAIWAN: TATUNG									
SHP-1	Mud volcano	MP	6.14	350	6.15	97.0	—	0.7040 ± 2	48
THC-2	Acid spring	AS	5.40	100	5.44	—	—		
DYK-1	Hell Valley	AS	4.40	5.7	5.00	96.0	41.1	0.7040	38
SCC-1	West End	AS	4.64	127	4.66	82.0	—		
CS-2	Chinshan	AS	3.93	4.5	4.77	72.0	11.9		
MTS-1	Central region	AS	4.35	950	4.35	92.0	680		
JAPAN									
CW-1	Hakone volcano	W	6.50	140	6.54	172	135	0.7040	
H-6	Hakone volcano	W	6.62	93	6.68	137	261		
H-9	Hakone volcano	W	5.99	18.2	6.28	144	161		
USU-2	Usu volcano	SF	6.15	236	6.16	736	—		
USU-3	Usu volcano	SF	6.07	125	6.10	236	—		
USU-15	Usu volcano	SF	6.19	107	6.23	666	—		
INDONESIA									
	Serua	SF	1.27	1.7	1.64	102	11.7	0.7084 ± 7	23
	Teon	SF	1.31	130	1.31	99	990	0.7076 ± 1	23
	Damar (400 m)	FF	1.69	600	1.69	135	2,630	0.7065 ± 1	23
	Damar (700 m)	SF	1.65	3,200	1.65	170	2,150	$0.7056^{\#}$	
	Sirung	SF	1.03	1.7	1.07	95	9.8		
	Lewotolo	SF	3.62	500	3.62	490	700		
	Lewotolo	SF	3.57	1,180	3.57	490	990		

The N_2/Ar ratio can often be used to estimate the proper correction factor when the correction is important. When a range is shown for R_C , the upper and lower limits are calculated from the air and solubility correction factors respectively.

‡ NC, Non-condensable gases.

§ C. J. Nye (personal communication).

|| Re-analyses of original gas samples from Craig *et al.*⁶ with Ne separation from samples and standards. R/R_A values are lower when Ne is removed from air standards: for example, the two Lassen Park analyses given here are 9% lower than the earlier values⁶ measured without Ne removal.

¶ Submarine basalts dredged by Stern from Mariana arc seamounts: Eifuku at $21^\circ 45' \text{N}$, $144^\circ 10' \text{E}$, and Ruby at $15^\circ 12' \text{N}$, $45^\circ 25' \text{E}$ on NSF-supported expedition: he also supplied the Sr isotope data.

J. C. Varekamp (personal communication).

borehole gases from the Marivalles geothermal field continue the consistency in $^3\text{He}/^4\text{He}$ ratio ($7.4 R_A$) for North American arc terrains.

Western Pacific arcs

Helium isotope ratios in the central Western Pacific arcs, the Marianas and Philippines, are very similar to the North American values. In the Mariana arc a hot spring on Agrigan volcano⁶ has a $^3\text{He}/^4\text{He}$ ratio of $7.4 R_A$, and phenocrysts from two submarine volcanoes¹⁴ have ratios of 7.2 – $7.65 R_A$. Volcanic gases from the Philippine arc have similar He signatures: $^3\text{He}/^4\text{He}$ ratios from Taal, Mt Pinatubo and Biliran Island range from 7.4 to $7.5 R_A$, identical to the Mariana and North American values. Lead isotope ratios in these volcanics also reflect a minimum contribution of subducted sediment¹⁵, although Sr isotope ratios are much higher than in western North American arcs¹⁶.

In the northwestern Pacific arcs, the Kurile–Honshu–Ryukyu system, a clear difference in the volcanic arc He signature is evident. In Japan, steam wells from the inner caldera of Hakone volcano have $^3\text{He}/^4\text{He}$ ratios of $6.5 R_A$, and high-temperature (700°C) fumaroles on Usu volcano in Hokkaido have similar ratios of $6.2 R_A$. These Japanese ratios are distinctly lower than the Aleutian and North American values, but very similar to values in the Kuriles and Kamchatka¹⁷, shown in Fig. 1. Recent work^{18,19} on Japanese hot-spring and volcanic gases shows a wide range in $^3\text{He}/^4\text{He}$ ratios, converging in the high-ratio, high-He samples to a statistical distribution with a mean $R/R_A = 6.5 \pm 1.5$, uncorrelated with He or air content. It seems likely that this range of He ratios simply represents analytical scatter about a mean volcanic ratio, $R/R_A = 6.5$, which agrees well with both our earlier⁶ and present data.

In northern Taiwan, gases from acid hot springs associated with Pleistocene volcanism in the Tatung region²⁰ range in $^3\text{He}/^4\text{He}$ ratio from 4.4 to $6.1 R_A$. The Tatung field lies near the intersection of the Ryukyu and Luzon arcs, ~ 150 km above the Benioff Zone defined by the subduction of the Philippine Plate beneath Eurasia²¹. Although the range in $^3\text{He}/^4\text{He}$ ratios indicates the presence of a radiogenic ^4He component, the highest ratio measured, $6.1 R_A$, is very consistent with the low $^3\text{He}/^4\text{He}$ ratios in the Kurile and Japanese volcanoes.

Indonesia

The most striking contrast to the relatively uniform $^3\text{He}/^4\text{He}$ ratios observed in the other arc terrains (6 – $8 R_A$) is the isotopic composition of He in the active volcanoes of the Banda arc in the eastern sector of Indonesia. The summit fumarole at Lewotolo, on the western edge of the Banda sector, has He with $R/R_A = 3.6$: eastward along this arc, three volcanoes have $^3\text{He}/^4\text{He}$ ratios as low as $R/R_A = 1.1$ – 1.6 . Although it is possible that addition of ^4He from circulating geothermal fluids is significant, it appears remote at Lewotolo and Damar ($1.7 R_A$) with summit fumaroles at 490°C and 170°C respectively. In two cases, Serua and Sirung, the correction for atmospheric helium is large, but the corrected ratios also point to a helium source for all Banda arc volcanoes that is much more radiogenic than in any other arc gases so far analysed. The Banda arc lavas show extreme enrichments in Sr, Nd and O isotope ratios, all suggesting a major input of crustal material^{22–24}.

Discussion

The data here show that our previous observation⁶ that active transport of mantle gases occurs in arc regimes is a general phenomenon in major volcanic arcs. In summit fumaroles uncontaminated by radiogenic ^4He from older country rock, the $^3\text{He}/^4\text{He}$ ratio is very close to that of mid-ocean-ridge basalts (MORBs) ($R/R_A = 8$). Except for the Banda arc, the result of a unique arc–continent collision, the uniformity in $^3\text{He}/^4\text{He}$ ratios contrasts markedly with the differences in tectonic setting and lava compositions of the arc systems. These range from the

continental arcs of western North America, where young crust is subducting into sediment-filled trenches, to the oceanic arcs of the Western Pacific, where old crust subducts into nearly sediment-free trenches. Andesites and dacites dominate the continental arcs whereas basalt and basaltic andesite are the major magma types in the oceanic arcs.

The arc helium signatures ($R/R_A = 6$ – 8) point to a MORB source dominating the production of arc lavas. Craig *et al.*⁶ observed that if arc volcanics were simply remelted downgoing oceanic crust, then very low $^3\text{He}/^4\text{He}$ ratios, $\sim 2 R_A$ or less, would be observed because of radiogenic ^4He production in old oceanic crust which should retain little if any primordial ^3He (ref. 8). Hydrothermal circulation also removes large quantities of ^3He from the oceanic crust. With a degassing rate of $4 \text{ atoms cm}^{-2} \text{ s}^{-1}$ ($1,000 \text{ mol yr}^{-1}$) of ^3He (ref. 25) and a formation rate of $3 \text{ km}^2 \text{ yr}^{-1}$ of oceanic crust, hydrothermal circulation will remove $> 10^{-5} \text{ cm}^3 \text{ s}^{-1}$ of helium to a depth of 5 km (ref. 8). Partitioning studies²⁶ show that $< 1\%$ of helium in a basalt lava resides in phenocrysts, so that cumulate gabbros do not hold ^3He . Direct measurement²⁷ of 100-Myr-old basalt glass shows low ^3He concentrations (0.5 – $0.2 \times 10^{-12} \text{ cm}^3 \text{ g}^{-1}$) with ratios $< 3 R_A$, because of radiogenic He production and diffusive losses⁹. These considerations all point to the conclusion that old oceanic crust is low in ^3He , has a $^3\text{He}/^4\text{He}$ ratio dominated by radiogenic ^4He , and cannot be the major source of helium in arc lavas.

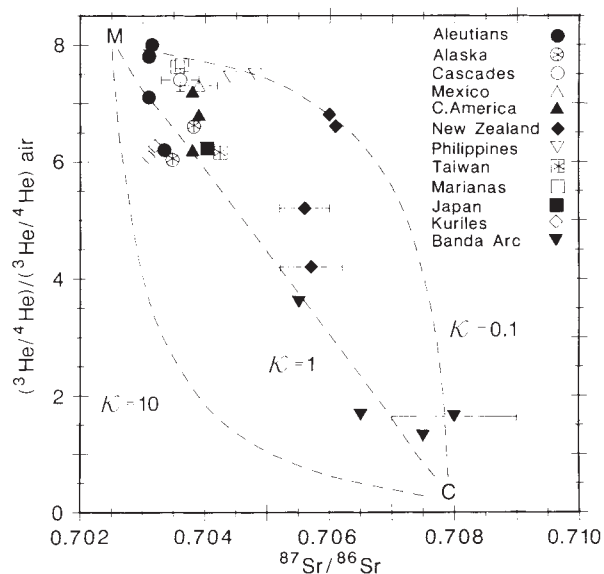


FIG. 2 A plot of $^3\text{He}/^4\text{He}$ against $^{87}\text{Sr}/^{86}\text{Sr}$ for 12 circum-Pacific arcs. The $^3\text{He}/^4\text{He}$ data are from this paper, refs 17 and 50 (Kuriles), and ref. 36 (SI0 data) and ref. 49 (New Zealand). $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for lavas from the same volcano as the helium data were used whenever possible; error bars on Sr ratios reflect the range measured at a volcano. The curves are mixing trajectories between a MORB component ('M' = $8 R_A$, 0.7025) and a crustal/sedimentary end member ('C' = $0.2 R_A$, 0.7080) defined by a parameter $\kappa = [(^3\text{He}/^4\text{He})_{\text{CRUST}} / (^3\text{He}/^4\text{He})_{\text{MORB}}]$. Sedimentary $^{87}\text{Sr}/^{86}\text{Sr}$ values are from refs 51 and 52. Note that whereas the $^3\text{He}/^4\text{He}$ ratio of component 'C' is fixed at ~ 0 (radiogenic He), the $^{87}\text{Sr}/^{86}\text{Sr}$ will vary for different sources (for example, 0.720 for old Precambrian crust), so that the curves for κ values are pinned at the 'M' composition, but move left or right for 'C' components with smaller or larger $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Thus the κ values for mixtures depend directly on the Sr isotope ratio assumed for 'C'. The He/Sr ratio of MORB is reasonably well constrained within a factor of two ($^4\text{He} \approx 10^{-5} \text{ cm}^3 \text{ g}^{-1}$, $\text{Sr} \approx 120 \text{ p.p.m.}$, $\text{He}/\text{Sr} \approx 3.2 \times 10^{-4}$ molar ratio). However, values of κ could vary by a factor of 100, from a minimum $\kappa \approx 0.1$ for pelagic sediments with very low He retentivity, to ~ 0.2 for old basaltic crust which lost all initial He at eruption, to $\kappa \approx 10$ for Mesozoic and older continental crust (2.7 p.p.m. U , 370 p.p.m. Sr). Thus an average 200-Myr-old crust which retained 50% of its radiogenic He might have a He/Sr ratio twice that of MORB.

Stern²⁸ discussed the similarities in $^{87}\text{Sr}/^{86}\text{Sr}$, K/Rb and K/Ba ratios in primitive arc volcanics and oceanic island basalts and suggested that an enriched source region produces arc lavas. However, we note that *no arc terrain has a $^3\text{He}/^4\text{He}$ ratio higher than that of normal mid-ocean-ridge basalt*. The $^3\text{He}/^4\text{He}$ ratios of oceanic islands fall into two groups relative to MORB helium²⁹: 'high- ^3He ' hotspots (for example Hawaii, Iceland, Samoa) have ratios significantly higher than MORBs^{9,29} with $R/R_A \approx 11$ –30, and could not be a source for arc magmas. However, the 'low- ^3He ' hotspots with $R/R_A \approx 5$ –8 (for example, Jan Mayen, Azores or Gough³⁰), do resemble arc magmas in their He signatures, and ratios of $^3\text{He}/^4\text{He}$ in fumaroles and of $^{87}\text{Sr}/^{86}\text{Sr}$ in lavas of the Aleutians and Cascades overlap the fields for Mohs Ridge and Azores Platform basalts^{31–34}. As with other geochemical tracers the relationships between $^3\text{He}/^4\text{He}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ do not provide a unique solution to the source for arc lavas, but it seems improbable that 'low- ^3He ' mantle source regions, principally found in the Atlantic Ocean²⁹, should exist beneath every circum-Pacific volcanic arc.

The $^3\text{He}/^4\text{He}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ distribution can also be produced by mixing a component from the subducting slab, rich in ^{87}Sr and ^4He , with a normal MORB mantle (He and Sr ratios of 8.0 – $8.5 R_A$ and 0.7024 – 0.7028 respectively). Because of the similarity in $^3\text{He}/^4\text{He}$ ratios between arc and MORB helium, it seems more probable that the source for arc volcanism resembles the MORB source in helium and that differences in $^3\text{He}/^4\text{He}$ ratios and other geochemical tracers such as $^{87}\text{Sr}/^{86}\text{Sr}$ result from a variable slab contribution from sediments or altered oceanic crusts.

Helium–strontium relationships

Figure 2 shows the $^3\text{He}/^4\text{He}$ data and measured or estimated $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for lavas from the same sites (Table 1) on ten circum-Pacific arcs, with trajectories calculated for mixing a MORB component (M) with a possible crustal or sedimentary source (C). Most of the data are consistent with the addition of up to ~25% of crustal He ($R/R_A = 8$ –6), and up to ~65% of the crustal Sr component, to a MORB source. That is, the values cluster in the region for $\kappa < 1$ (see Fig. 2 legend), so that the fraction of mantle He in arc lavas is equal to or greater than the fraction of mantle Sr, consistent with a lower He/Sr ratio in the crustal component. The 'mixing curves' are of course subject to considerable uncertainties inherent in representing two very different elements such as He and Sr. Fumaroles on two andesitic volcanoes (Ngauruhoe, 516°C ; White Island, 540°C) in New Zealand³⁶, and two volcanoes (Pinatubo and Taal) on the West Philippine (Manila) arc, lie on the minimum κ curve ($\kappa = 0.1$), suggestive of a MORB source diluted with a low He/Sr crustal component, possibly subducted pelagic sediment with a high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and Sr content.

Contamination of arc magmas during ascent through continental crust does not seem to have a large effect on $^3\text{He}/^4\text{He}$ ratios. Because the U/Sr ratio is higher in crustal rocks than in MORB, pre-Cenozoic rocks that retain helium should have He/Sr ratios greater than MORB ($\kappa > 1$) and on mixing with arc magmas should produce large changes in $^3\text{He}/^4\text{He}$ ratios relative to $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. In the Alaskan arc (Fig. 1) $^3\text{He}/^4\text{He}$ ratios in the continental volcanoes of Mts Griggs, Douglas and Augustine are not lower than those of summit fumaroles in the Aleutian arc: indeed Mt Douglas ($R = 7.9 R_A$) has the highest $^3\text{He}/^4\text{He}$ ratio in the arc. Thus the influence of the Mesozoic continental crust on the $^3\text{He}/^4\text{He}$ ratios of these magmas seems to be less than a 5% dilution. Sr isotope ratios support this conclusion, as no systematic gradient exists from the Aleutian to the Alaskan sectors^{32,35}. However, $^3\text{He}/^4\text{He}$ ratios for the two easternmost volcanoes in the chain, Mt Spurr ($6.6 R_A$) and Mt Wrangell ($6.1 R_A$), are significantly lower (Fig. 2, on the $\kappa \approx 1$ mixing curve), very probably because of dilution with helium from a thicker continental crust.

The Banda arc data lie at the opposite extreme in Fig. 2, with

a crustal component amounting to ~80% for He ($R/R_A = 1.6$), and ~80–90% for Sr based on a 0.7080 end member. If, however, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the crustal end member is closer to 0.720 (refs 23, 24), as expected for the Precambrian Australian craton, the data would require $\kappa \approx 5$ for a mixing curve, corresponding to a helium-rich crustal component. In fact the He/Sr ratio in Precambrian rocks is probably at least ten times the MORB ratio, and this end member would also fit the correlation between $\delta^{18}\text{O}$ and $^{87}\text{Sr}/^{86}\text{Sr}$, which suggests a 25% sialic contribution^{23,24}. Conversely, the Sunda arc lavas in the western Indonesian sector, with Sr ratios²³ of $\sim 0.7048 \pm 0.0004$ derived from subduction of oceanic crust, are predicted (Fig. 2) to have $^3\text{He}/^4\text{He}$ ratios of $R/R_A = 7 \pm 0.5$, with a well defined transition to the much lower Banda arc ratios near Lewotolo ($R/R_A = 3.6$).

Regional variations

Considering the $^3\text{He}/^4\text{He}$ ratios in summit fumaroles, phenocrysts and least radiogenic samples, six volcanic arcs in Fig. 1 (the Aleutian–Alaskan arc, Cascades, Trans-Mexican belt and the Central American, Mariana and Philippine (east and west) arcs), have essentially indistinguishable mean ratios with $R/R_A \approx 7.5 \pm 0.5$, corresponding to a mantle He fraction $X_M \approx 94 \pm 6\%$. In the North Pacific only the Kurile–Honshu–Ryukyu arc system, including Kamchatka and the Kuriles, Japan and Taiwan, has clearly lower He ratios, with a mean value of $R/R_A \approx 6.4$, and a mantle He fraction of ~80%. Although each of these arcs has a distinct geochemical signature which may reflect the composition of the mantle wedge or the age and composition of the overriding or subducting crust, no systematic correlation between these variables and the $^3\text{He}/^4\text{He}$ ratios exists. Thus the Mariana arc, where the oldest oceanic crust (Jurassic) is subducting, has among the highest He ratios, $R/R_A = 7.6$, like the Philippine and Aleutian arcs where considerably younger crust is subducted. Most of the data points in Fig. 2 require a helium-poor component with a low He/Sr ratio ($\kappa \approx 0.4$), either sediment or altered oceanic crust, mixing with a MORB source. Radiogenic production of ^4He during 100 Myr with 0.1 p.p.m. of U should produce a He/Sr ratio of ~0.2 times the MORB ratio if no He is removed, but because of the mobility of He and the lack of data on crustal He/Sr ratios, it is difficult to predict the nature of the 'C' component in individual cases.

Helium in arcs associated with subducting oceanic crust is almost entirely derived from a dominant MORB component in the mantle wedge, but we do not know at present whether the subducting crust is almost totally depleted of both ^3He and ^4He , or whether radiogenic He is still present in significant amounts but is not mobile. In the Banda arc of Indonesia most of the volcanic arc He (80%) is derived from subducting continental crust associated with a Precambrian craton, so that here at least a substantial increment of ^4He relative to the He derived from the mantle wedge is retained during subduction and then mobilized into the erupting arc magma. Despite our uncertainties as to the processes actually responsible for cooking the final $^3\text{He}/^4\text{He}$ recipes for arc volcanic gases and lavas, ^3He , because it has essentially zero concentration in the oceanic and continental crust, is the most powerful tracer we have for studying the crustal contribution to arc magmas. □

Received 2 November 1988; accepted 22 February 1989.

1. Ringwood, A. E. *J. geol. Soc. Lond.* **130**, 183–204 (1974).
2. Marsh, B. D. & Carmichael, I. S. E. *J. geophys. Res.* **79**, 1196–1206 (1974).
3. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 743–746 (1976).
4. Kay, R. W. in *Island Arcs, Deep Sea Trenches, and Back-Arc Basins* (eds Talwani, M. & Pitman, W. C.) 229–242 (American Geophysical Union, Washington, DC, 1977).
5. Gill, J. B. *Orogenic Andesites* (Springer, New York, 1981).
6. Craig, H., Lupton, J. E. & Horibe, Y. in *Terrestrial Rare Gases* (eds Alexander, E. C. & Ozima, M.) 3–16 (Japan Sci. Soc. Press, Tokyo, 1978).
7. Rison, W. & Craig, H. *Earth planet. Sci. Lett.* **66**, 407–426 (1983).
8. Lupton, J. E. & Craig, H. *Earth planet. Sci. Lett.* **6**, 133–139 (1975).
9. Craig, H. & Lupton, J. E. *Earth planet. Sci. Lett.* **31**, 369–385 (1976).
10. McCullough, M. & Perfit, M. *Earth planet. Sci. Lett.* **56**, 167–179 (1981).

11. Brown, L., Klein, J., Middleton, R., Sacks, I. S. & Tera, F. *Nature* **299**, 718–720 (1982).
12. Jacob, K., Nakamura, K. & Davies, J. N. in *Island Arcs, Deep Sea Trenches, and Back-Arc Basins* (eds Talwani, M. & Pitman, W. C.) 243–258 (American Geophysical Union, Washington, DC, 1977).
13. Fournier, R. O., Hanshaw, B. B. & Urrutia Sole, J. F. *Trans. Geotherm. Resources Council* **6**, 89–91 (1982).
14. Stern, R. J., Bloomer, S. H., Lin, P.-N., Ito, E. & Morris, J. *Geology* **16**, 426–430 (1988).
15. Mukasa, S. B., McCabe, R. & Gill, J. B. *Earth planet. Sci. Lett.* **84**, 153–164 (1987).
16. Knittel, U. & Defant, M. J. *Earth planet. Sci. Lett.* **87**, 87–99 (1988).
17. Baskov, Y. et al. *Geochem. Int.* **10**, 130–138 (1973).
18. Sano, Y. & Wakita, H. *J. geophys. Res.* **90**, 8729–8741 (1985).
19. Nagao, K., Takaoka, N. & Matsubayashi, O. *Earth planet. Sci. Lett.* **53**, 175–188 (1981).
20. Wang, Y. *Proc. geol. Soc. China* **13**, 41–55 (1970).
21. Katsumata, M. & Sykes, L. R. *J. geophys. Res.* **74**, 5923–5948 (1969).
22. Whitford, D. J. *Geochim. cosmochim. Acta* **39**, 1287–1302 (1975).
23. Whitford, D. J., White, W. M. & Jezek, P. A. *Geochim. cosmochim. Acta* **45**, 989–995 (1981).
24. Margaritz, M., Whitford, D. J. & James, D. E. *Earth planet. Sci. Lett.* **40**, 220–230 (1978).
25. Craig, H., Clarke, W. B. & Beg, M. A. *Earth planet. Sci. Lett.* **26**, 125–132 (1975).
26. Kurz, M. D. & Jenkins, W. J. *Earth planet. Sci. Lett.* **53**, 41–54 (1981).
27. Takaoka, N. & Nagao, K. *Init. Rep. DSDP Legs 51 and 52*, 533 (1980).
28. Stern, R. J. *Yb. Carnegie Instn Wash.* **80**, 455–462 (1981).
29. Craig, H., Rison, W. & Poreda, R. J. *Eos* **66**, 1079 (1985).
30. Kurz, M. D., Jenkins, W. J., Schilling, J.-G. & Hart, S. R. *Earth planet. Sci. Lett.* **58**, 1–14 (1982).
31. Church, S. E. & Tilton, G. R. *Bull. geol. Soc. Am.* **84**, 431–454 (1973).
32. Kay, R. W., Sun, S. S. & Lee-Hu, C. N. *Geochim. cosmochim. Acta* **42**, 263–273 (1978).
33. Poreda, R., Craig, F. & Schilling, J.-G. *Earth planet. Sci. Lett.* **78**, 1–17 (1986).
34. Wagoner, D. G. & Schilling, J.-G. *Eos* **64**, 346 (1983).
35. Arculus, R. J., DeLong, S. E., Kay, R. W., Brooks, C. & Sun, S. S. *J. Geol.* **85**, 177–186 (1977).
36. Torgeson, T., Lupton, J., Sheppard, D. & Gigenbach, W. J. *Volcan. geotherm. Res.* **12**, 283–294 (1982).
37. Gorskov, G. S. *Volcanism and the Upper Mantle* (Plenum, New York, 1970).
38. Fujimaki, H. *Lithos* **19**, 129–140 (1986).
39. Dixon, T. H. & Batiza, R. *Contr. Miner. Petrol.* **70**, 167–181 (1979).
40. Craig, H., Lupton, J., Welhan, J. & Poreda, R. *Geophys. Res. Lett.* **5**, 897–900 (1979).
41. Myers, J. D., Marsh, B. D. & Sinha, A. K. *Contr. Miner. Petrol.* **91**, 221–234 (1985).
42. Peterman, Z. E., Carmichael, I. S. E. & Smith, A. L. *Bull. geol. Soc. Am.* **81**, 311–318 (1970).
43. Hedge, C. E., Hildreth, R. A. & Henderson, W. T. *Earth planet. Sci. Lett.* **8**, 434–438 (1970).
44. Moorbath, S., Thorpe, R. S. & Gibson, I. I. *Nature* **271**, 437–438 (1978).
45. Rose, W. I. Jr. et al. *J. Geol.* **85**, 63–88 (1977).
46. Pushkar, P. J. *Geophys. Res.* **73**, 2701–2713 (1968).
47. Thorpe, R. S., Francis, P. W. & Moorbath, S. *Nature* **277**, 44–45 (1979).
48. Shih, C. Y. *Eos* **54**, 132 (1973).
49. Ewart, A. & Stipp, J. J. *Geochim. cosmochim. Acta* **32**, 699–736 (1968).
50. Bailey, J. C., Larsen, O. & Frolova, T. I. *Contr. Miner. Petrol.* **95**, 155–165 (1987).
51. Woodhead, J. D., Harmon, R. S. & Fraser, D. G. *Earth planet. Sci. Lett.* **83**, 39–50 (1987).
52. von Drach, V., Marsh, B. D. & Wasserburg, G. J. *Contr. Miner. Petrol.* **92**, 13–34 (1986).

ACKNOWLEDGEMENTS. We thank R. Motyka, J. Welhan, J. Varkamp, R. Poorter, S. Arnorsson, J.-L. Cheminee, F. Vidal, Y.-C. Chung, S. Matsuo and the late D. Johnston, who collected samples for us, often under hazardous conditions. R. Motyka provided logistical support to R.J.P. in the Aleutian and Y. Horibe worked with H.C. in the gas collections from Hakone and Usu volcanoes. We thank J. Urrutia and the INDE in Guatemala, F. Vidal and S. Mercado of the Instituto de Electricas in Mexico, and T. Powell of UNOCAL and P. Malixi and PNOC in the Philippines, for field assistance and logistical support. This research was supported by the Volcanology and Mantle Geochemistry Program of NSF.

The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase

Frank Sprenger, Leslie M. Stevens & Christiane Nüsslein-Volhard

Max-Planck-Institut für Entwicklungsbiologie, Abteilung Genetik, Spemannstr. 35/III, D-7400 Tübingen, FRG

The maternal gene *torso*, required for determination of anterior and posterior terminal structures in the *Drosophila* embryo, was cloned using P-element tagging. Genetic evidence suggests that the action of the gene product is spatially restricted to the terminal regions; the *torso* messenger RNA, however, is evenly distributed. Structural similarities of the predicted *torso* protein with growth-factor receptor tyrosine kinases suggest that the spatial restriction of *torso* activity results from a localized activation of the *torso* protein at the anterior and posterior egg pole.

EARLY pattern formation in the *Drosophila* embryo depends upon maternal gene products provided during oogenesis and deposited in the freshly laid egg. For the determination of the anteroposterior axis of the embryo three classes of maternal genes have been identified: the anterior, the posterior and the terminal class¹. Each class defines a pattern-forming process which results in the localization and/or activation of at least one maternal gene product which is necessary for the appropriate spatial expression of the zygotic target genes. In addition to the anterior and posterior genes, which function through localized components, a third organizing activity is required for the formation of the unsegmented terminal regions. At least six maternally expressed genes are necessary for the formation of the most anterior and posterior regions of the larva, acron and telson (as defined in ref. 1): *torso*(*tor*)², *trunk*(*trk*)², *torsolike*(*tsl*) (H.-G. Frohnhofer, personal communication), *f(1)Nasrat* (*fs(1)N*)³, *fs(1)polehole* (*fs(1)ph*)⁴ and *l(1)polehole* (*l(1)ph*)⁵. Embryos derived from mothers homozygous for mutations in this terminal class of genes fail to develop the labrum and the head skeleton is reduced in size. In addition, all elements posterior to the seventh abdominal segment are absent, including

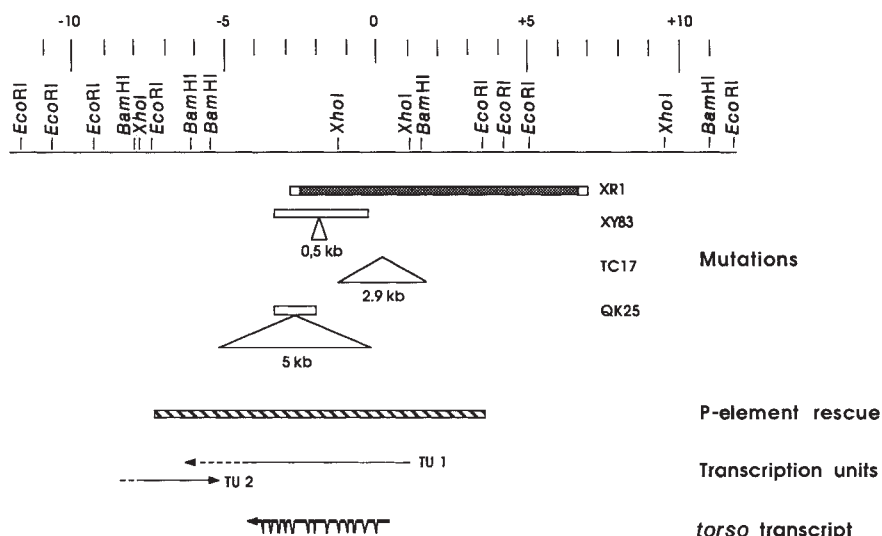
the eighth abdominal segment and the telson. Mutations in any gene of the terminal class give an identical phenotype, which suggests that these genes are part of a single pattern-forming process that results in the spatially restricted expression or in the activation of zygotic terminal genes such as *tailless*(*tll*)^{6,7}. In this pathway *torso* appears to play a crucial part, which is suggested by the phenotype of *torso* gain-of-function alleles. These mutations produce a phenotype opposite to the loss-of-function alleles⁷: elements of the termini are formed but the segmentation of the thorax and abdomen is suppressed. However, segmentation is restored in embryos which are in addition mutant for a null allele of *tll*. This indicates that the function of the *torso* product is mediated via *tll*, and suggests that in embryos of *torso* gain-of-function alleles, *tll* is expressed everywhere as a result of an ectopically active *torso* product. In the wild-type embryo the locally active *torso* product therefore provides cues required for correct spatial activity of terminal zygotic genes such as *tll*. Because cytoplasmic transplantation experiments revealed no localization of wild-type *torso* activity, it has been suggested⁷ that the spatial restriction of *torso* activity may arise from a localized activation of the *torso* product through a modification mechanism by a preexisting spatial signal, which might be provided by one of the other terminal genes.

Here, we report that the product of the *torso* gene has structural similarities to receptor tyrosine kinases. This suggests a mechanism of the restricted *torso* action and provides a molecular explanation for the gain-of-function phenotype.

Molecular cloning

The *torso* locus maps to the right arm of the second chromosome at cytogenetic position 43D3-E7. DNA from the *torso* locus was isolated by the P-element transposon tagging method⁸ from *tor*^{TC17}, a P allele obtained from T. Schüpbach. A genomic library was constructed from a subline of *tor*^{TC17} in which many of the original P elements (over 50) had been removed by recombination. Two overlapping λ clones were isolated, which both contained 2.9 kilobases (kb) of P-element sequences and hybridized at cytological positions 43 E5-11 to wild-type salivary

FIG. 1 Structure of the genomic *torso* region. Numbers above the restriction map refer to distances in kb, the 0-point is defined by the P-insertion site. Mutations were mapped using Southern blot hybridization (tor^{XR1} , tor^{XY83} , tor^{QK25}) and DNA sequencing (tor^{TC17}). tor^{TC17} was isolated in a hybrid dysgenic cross (T. Schüpbach, personal communication), tor^{QK25} is an EMS-induced allele isolated by Schüpbach and Wieschaus², tor^{XR1} is an X-ray-induced revertant of tor^{RL3} (ref. 7) and tor^{XY83} is an X-ray-induced revertant of tor^{Y9} (ref. 7). Embryos derived from mothers homozygous for these alleles develop a strong *torso* phenotype but show no other detectable phenotype. The filled bar represents the deleted region in tor^{XR1} , open bars above the triangles represent wild-type restriction fragments in which mutation-induced size changes were detected by Southern hybridizations. The 12-kb *EcoRI* fragment sufficient for P-element-mediated rescue is shown as a hatched bar. The two transcription units and their direction of transcription are presented as arrows. The exon-intron structure of the *torso* transcript, as inferred from the cDNA clone pCD7, is shown.



gland chromosomes. Fragments of these clones lacking P-element sequences were used to isolate λ clones from an Oregon P2 library, covering ~12 kb of DNA on either side of the P-insertion site (Fig. 1).

When the DNA structure of 35 ethyl methane sulphonate-induced and X-ray-induced alleles was analysed, three showed altered restriction maps in the cloned region as detected by Southern-blot hybridizations. The alleles tor^{QK25} and tor^{XY83} contain insertions of 5 kb and 0.5 kb, whereas the allele tor^{XR1} has a 9.5-kb deletion (Fig. 1) suggesting that this region contains sequences from the *torso* locus.

To confirm that we had cloned all of the sequences necessary for *torso* function, we used P-element-mediated germ-line transformation to rescue *torso* mutants. A 12-kb *EcoRI* fragment was cloned into the P^{PA} vector⁹, which carries the gene encoding alcohol dehydrogenase (*Adh*) as a marker and was injected into embryos from a strain mutant for both, $tor(tor^{WK})$ (ref. 2), and *Adh* (Adh^{H2}). Eleven independent transformant lines were obtained, all of which rescued both the ethanol sensitivity and wild-type *torso* function.

The *torso* transcript

This 12-kb *EcoRI* fragment was used to screen a *D. melanogaster* embryonic complementary DNA library¹⁰. Two classes of cDNAs were isolated corresponding to two transcription units which are transcribed in opposite directions (TU1 and TU2) (see Fig. 1). Northern analysis revealed that the mRNA corresponding to TU2 is constitutively expressed during development (data not shown). Because only a portion of this transcription unit is contained within the rescuing *EcoRI* fragment, it is unlikely to represent the *torso* transcript.

Overlapping cDNA clones were isolated from TU1. The longest of these, pCD7 (3,063 base pairs (bp)), was used to examine the temporal expression pattern of the corresponding RNA on northern blots (Fig. 2a). A transcript of ~3.6 kb can be detected in RNA from 0–4-h-old embryos. After 4 h of development this signal is greatly reduced, but a very low level of expression is detectable throughout development after longer exposures. Adult females, but not males, show a high level of this transcript, consistent with expression in the ovary. In addition, a minor 5.5-kb transcript is visible after longer exposures (Fig. 2b). Neither the 3.6 nor the 5.5-kb transcript can be detected in RNA from the deficiency tor^{XR1} (Fig. 2b), indicating that both transcripts are derived from the *torso* region. Further analysis with smaller probes revealed that the 5.5-kb transcript is a 3'-extended version of the 3.6-kb transcript, suggesting that this minor transcript is the result of inefficient 3' processing of

the original transcript. Because TU1 has a pattern of expression that is consistent with a maternal gene, and because it is the only transcription unit entirely contained within the rescuing 12-kb fragment, we conclude that TU1 is the *torso* transcription unit.

Expression of *torso*

The distribution of the *torso* transcript in ovaries and early embryos was examined by *in situ* hybridization to tissue sections. The transcript can be detected at low levels in the earliest stages of oogenesis (germarium, data not shown). It accumulates in the nurse cells until oogenic stage 10, with relatively low levels of transcript detectable in the oocyte (see Fig. 3a). After stage 11, when the nurse cells transport their contents into the oocyte very rapidly, the *torso* mRNA becomes evenly distributed within the oocyte and the nurse cells (data not shown). Immediately after egg deposition during early cleavage stages (Fig. 3b), the transcript is uniformly distributed in the embryo. During

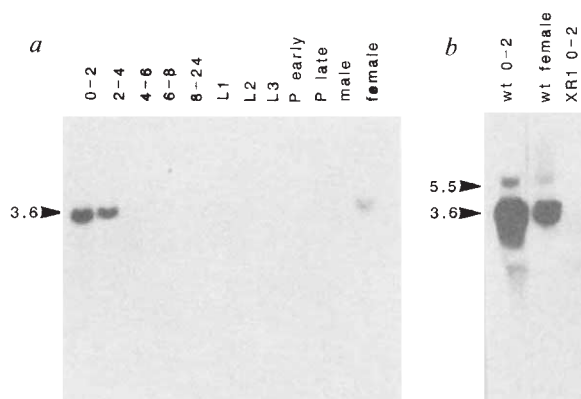


FIG. 2 Northern blot analysis. a, Developmental profile of the *torso* transcript. For the developmental stages indicated, 20 μ g total RNA were analysed by northern blot hybridizations. Abbreviations: L: larval stages, P: pupae. For size calibration, an RNA-Ladder (BRL, Bethesda) was used. b, Over-exposure of a northern blot containing wild-type and tor^{XR1} total RNA. Each lane contains 20 μ g total RNA.

METHODS. RNA was run on 1% agarose gels containing formaldehyde and transferred to Hybond nylon filters (Amersham, UK). Hybridization was performed according to the manufacturer's instructions, using nick-translated pCD7 DNA as a probe. RNA isolation and other standard techniques were as described³⁴.

syncytial blastoderm (Fig. 3c) the RNA moves towards the periphery of the embryo, apparently accompanying segregation of cytoplasm from yolk material. At the end of syncytial blastoderm, most of the *torso* RNA is found in the cytoplasm underneath the nuclei. The intensity of the label decreases with increasing age of the embryo and after cellularization no signal above background can be detected. The distribution of the *torso* transcript resembles that seen for total poly(A)⁺ RNA¹¹ and shows no apparent localization. The observed signal is specific, however, because embryos and oocytes from homozygous *tor*^{XR1} flies lack any signal above background levels (Fig. 3b).

The *torso* gene and protein structure

We have sequenced both the 3.1-kb cDNA and approximately 4.7 kb of the genomic *torso* region (Fig. 4). The cDNA is truncated at both the 3' and 5' ends, but appears to contain the entire coding sequence (see below). The sequence ATCATTC, which fits the heptanucleotide consensus sequences ATCA(G/T)T(C/T) found at the 5' end of many *Drosophila* mRNAs (ref. 12), lies 105 bases 5' to the beginning of the cDNA sequence, suggesting that this may be the 5' end of the mRNA. In addition, a consensus TATA and CAAT-box (ref. 13) are 26 and 79-bp upstream from the presumed transcription start site. A consensus polyadenylation signal AATAAA, which is normally present some 10–30 residues upstream of the poly-A tail¹⁴ directly follows the 3' end of the cDNA. The deduced length of the *torso* mRNA is therefore 3.2 kb, which is in good agreement with the 3.6 kb determined from gel migration and assuming a poly(A) tail of some 100 bases. Comparison of the cDNA and genomic sequences indicates that the *torso* gene contains at least 13 introns, which are, from 5' to 3', 67, 152, 60, 56, 68, 56, 57, 61, 67, 54, 60, 56 and 58 bp long.

Conceptual translation of the complete cDNA sequence in all three reading frames reveals a single long open reading frame of 2,936 bp that is preceded by an in-frame stop codon and

terminated with two stop codons. Within the first 82 codons after the stop codon which defines the start of the open reading frame there are three ATGs. Only the first exhibits a match to the consensus translation initiation site described for *Drosophila*¹⁵. Starting with the first Met, the open reading frame consists of 2,768 base pairs, encoding a 923 amino-acid (aa) protein with a predicted relative molecular mass of 105,162.

A hydropathy profile¹⁶ of the predicted polypeptide (Fig. 5) displays a strongly hydrophobic region of 22 amino acids between residues 400 and 422. By the criteria of Klein *et al.*¹⁷, this hydrophobic segment has a high probability of spanning a membrane. This segment is preceded by a positively charged lysine residue that could interact with the polar groups of membrane lipids and is followed by two charged arginines that could serve as a transfer-stop signal. There are 15 potential glycosylation sites found in the sequence, twelve of which are amino-terminal to the hydrophobic segment. A shorter hydrophobic region of 17 amino acids is present at the amino terminus of the protein and exhibits several features typical for leader sequences which are important for transport into the endoplasmic reticulum (ref. 18). A possible cleavage site for the signal peptidase, deduced from the $-3/-1$ rule¹⁹, is between residues 20 and 21. These features of the primary structure suggest that the *torso* protein is a transmembrane protein consisting of an extracytoplasmic domain of 380 amino acids, a transmembrane domain of 22 amino acids and a cytoplasmic domain of 402 amino acids.

A putative receptor tyrosine kinase

Comparison of the *torso* polypeptide with published sequences indicates significant homology between *torso* and proteins with tyrosine kinase domains. Alignment of the *torso* protein sequence with other protein tyrosine kinases reveals a high degree of amino-acid identity over the entire catalytic domain (Fig. 6), including the invariant lysine which is believed to be

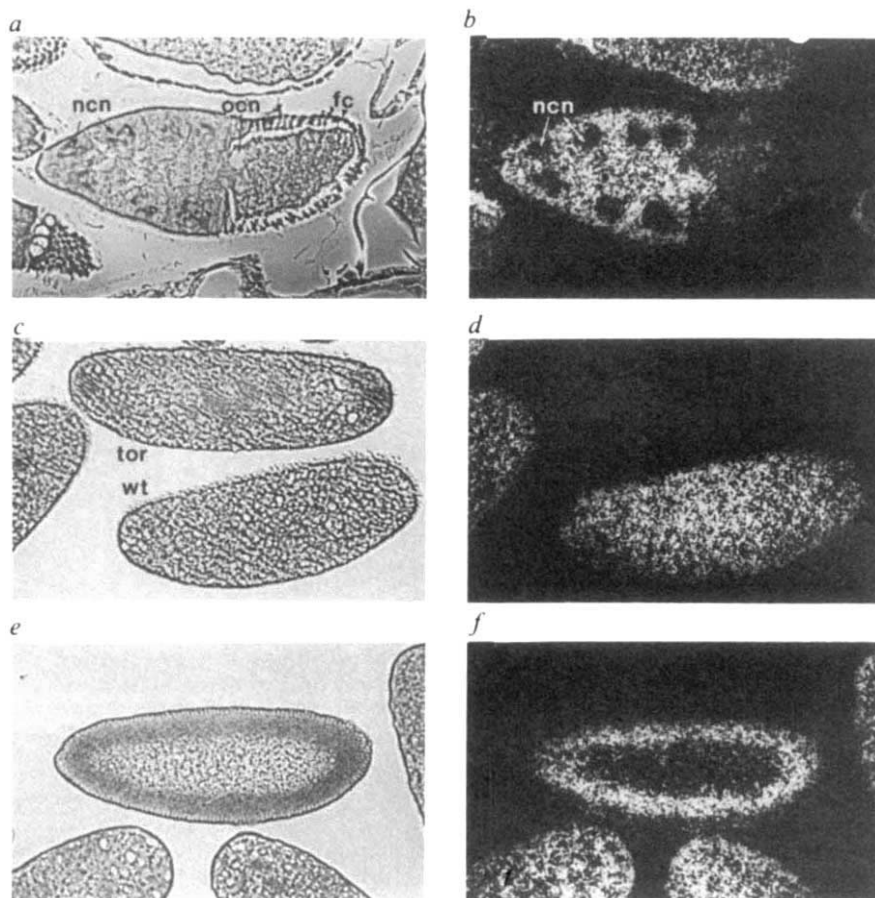


FIG. 3 Spatial pattern of the *torso* transcript during oogenesis and early embryogenesis. *a* and *b*, Late stage-10 follicle. The nurse cell complex is to the left, the oocyte in the right half of the egg chamber. The dorsal side is up. No labelling can be detected in the follicle cells and the oocyte nucleus. *c* and *d*, Early cleavage (<2 h) wild-type (*wt*) and *tor*^{XR1} (*tor*) embryo. *e* and *f*, Syncytial blastoderm embryo (<4 h). Most of the label is in the cytoplasm below the nuclei. No label was detected at any oogenic or embryogenic stage in sections from homozygous *tor*^{XR1} females (only shown for early cleavage stage). Abbreviations: ocn: oocyte nucleus, nc: nurse cell nuclei, fc: follicle cell.

METHODS. Preparations of tissue sections and hybridization conditions using ³⁵S-labelled pCD7 derived antisense RNA were as described³⁵. Embryos and ovaries were treated identically, except devitelinization was omitted for ovaries. Oogenesis stages are those described by King³⁶.

1 tccatgttgagtggtttcgactactcgaatcgatcgatgcaacaacttgatgcaccaatgtaatcgagcggttctatcagaaataacctgaatcagagcgcaactgag
101 gtcactgtgcattttgcaagtcgctatcgaagaaataacctgtatgttcagctctggtccaaatgaaatggaatcagggaaagtgtgatcagcgaataagctgata
201 atgtggggcgaataatgcatagatctgttttttgggttaatttcgaataatggcgcccaactgggtttctatagggtggaagcgaccagcactgactctgtgatt
301 atcgggtttgctgcaatacaagctcttttggcgcaacagatcatagaaattgaaatacaaacggttggtggtggtatcacaaatgaaatttgcgaagaggtt
401 atctgaagtcaatgttctatgcttatcccaagctcgccgctactatcagggatagcagatccaaatttttgcatttcaaatgacccctatgcattccatccc
501 atccatcagatcaagtaatacattcgatcgagtcgaagagtgaaaaacaaatacaaaaacaaactgagcgaaattatatcgaagataccgcgctctctcatccc
601 tggctcctctcttcgctgcttttttaccatcgacttttttggcctcagttttctcagtttctcagtttctcagtcaggaagataatccggaagaagagcgcggtattg
701 atCTCGAAAGTCTCGAAGCTTAGAAGAACTAAGATTTTCGAATTTTGGCTGTGTGTGTTTCTGCTGTTGTATCAAGGTGGCTGGTGGCAAAAGTGCATAAACTTT
801 AAGTCGGTGTTTAAACAAATACACATTTTCTTCCACACACACACACCCGACACACGAGAACTAAAGAGAGGTTTCAAAGAGCAGAACGAAAGAAATGCG
901 TATTTTCTATGCGAAGTACGCATTATTTCTTCTGGTTTTTCTGGGAAGCAATCAAGGTGAAATGTTGCTAATGGACAAAATCTCTCAGCATTAAGACGCT
1001 TCTCAAGCTACCCGCTGTCACCCAGAATTTCTGGAAGAGGCGGATGgtattgttacaccttagaagtgtggaacactccatacccaaaagtggatg
1101 cttctttttctcgacagAGTTTCCGAAGCTCTTTAAAGAGCTCAGAGTTAATGGAACATTTCCCGGGGCTTCCGCAAGGTGCAGGAAATACACAGATG
1201 AACATGATCTCGCCGACCGGAGTCGGAATCGTTTTCCAAATGATTTGGGTGCAGCAGCAGGCGGACCGGCTCCAAATGCCCACTACATATCC
1301 GGGTGGATGCTGTCAAGGACGACAAACAAAGAAACACCGCTTTTACCTGgtcagatatttagatgtaaacctcattactatttcttataaataccattttatt
1401 tgatgaattttctagaaaaagatgtactatgtagtcatacaagacctaatgttttcgttttacaagtggaatcgctcttttctcagctacttctcgtctgcagT
1501 CTGATGACAACCTTCTCATCTCTGCGGGATTTGGAGTCCAACCTTACCACAAACATACCCGCGCTGGCGATGCACGGAGTGGGACGATCTCTTGATAG
1601 AAAGGACGACGAGCTTCGCCACCCCTCATCCGAGGCTATCAGCCGACGAAATGGGAGCGGTGAATCTGCTGGGTTTGTCCCCAACGACGACGCTGCAT
1701 CACATTTGCTGCCGAAATCGAGTGGAAAGCCATCGCGGGtagtagtccatcagtttaataatcaatcaacgatctacgaacctacttctcatattccagAG
1801 GCAACTGCTATTTCGACATGGTGTCTGATTCAACCAACACAGCGTGAATATGGACGAGCCACTGGAGGTGCAGTTCCGGGATgtgagttgcaatgcaagccc
1901 ctgcagctcagtagcatagctaatcttttaactgcagCAAAAAGCTGTACGAGCAGCGGTGCACAACTTTGAGTTTGACAACAGTATCACGTTGGCG
2001 TAAGAACCGGTGAACATAATGAATCGATCGAGAGCGGATCTGCAGTGGCTGCCAATCGCTGTTCGAAGCTGCTTGAGTTGTATCCCTATACATACACAT
2101 CTGCGtagtggttcctcgaaagcctataataataataataataattgttccacctttctatagCACCCATAAGCCAGAGAATTTTACTG
2201 TGACCCAGAAGCATCTGCCAAATATTTGGCCCTGAACATCACTGGCGCGCTCCAGATACCTGGCCGATTAACATACACTTCACATCTTGATCT
2301 ATTCAAGAGGAGTACGGAGTCTAACTATACACTTGACCAAAACAGGACCCACTCTATGTACCCAAAGTACCGGTACTGGGTTCCCAATTCGAAGTACAT
2401 TTGGTGGCCGAGTCGGCAGCGGGAAGAACTCTCGGTTTGACGTTTGACCAAGGTTCTCTGAGGTTGTGTTGCTGAGCGgtacgtacgctgaaggaatt
2501 aaatatttagacatccatttccatccattgacagAGGGCAACATGGTCAAGTTGGTCACTTTTATTATCTGCGCAATGTGCTGCAATTTTGATGCTGGC
2601 TCCCTGACGTTCTGCAGACGAATCGTTCGAGGTTTCAGCGGCTGCAATGGACGCTAAGGACGCGGAAGGCAAGTGAATTTCTATCTCTCCCTGATGGACA
2701 CGAGTGGCTGCTGTTCACTCCCTCTCGGCCAAGCAGAGTCTGGAAGTAATGCAGCAGCTGGAGTGGAGCCACACTCGGTGCTCTCAGGATGTCTCGG
2801 CGAAGGAGCCTTTGGCTTGGTGCAGCTGGAGTTTACAAGAACCGCAAGTGGCGCTCAAGTTGCTGAAAGgttgtaaccatttggatttgaagtccttga
2901 ataattctggaatttttggttctcagatTGAACCAACACGAGGAGCGTATATGCGTTCAAGTGCGAATTCAGATGCTCAAGCGGCTGGGCAAGCATC
3001 CAAATATTGGGGTATCGTGGGATACCTCCACTCGTTTGTAGCAACAGATGTTGCTAATTTGAATCTGCAGCCTTGAAGCCTGCAGAACTTTTACG
3101 gtaagttatttttatacatatttggaaaacagtgatgtatgtaaaaatgtattacttagTCAGGAGTGAAGTTTACGCGAGGACGAAATGCAATTGG
3201 ACTTAAGAAGAACCTTGAACAGACGCTGGACACACCGAGCGTTTAAACCGACTCCCTAGAAATTCATCTCATGATGCAGATATCAACAACTCGATG
3301 CTGCTCACTGGAAGAGGAGTGAATCGGATCAGACACACTAAGTGCATGTGAGACTACACCTCATCGAATACCAATGCAGCGGACAAACGAG
3401 GCTATGGCCTGGGAGACATTGAAACACTCGGTGGGAGTTACATTTCCAAACCGCTGAAAGCTCAAGAGTACGCCAAAACGCGAAGCTGAAGCGGACGC
3501 CAAGAAGACTCGAAGCAGGATTTCAAATCGCACAAACGAAGACGAACTTTTGAGAACAAGGAATCACTTTGATTGCTTGACTCATCGGATACCAAGCC
3601 CGAATACCACTGAAATATGCAGATTGCTAGACTCGCCCAACAGGTGGCGGTGGGAATGgtagcctacatctaatctatccctaaatgtaattaaaaag
3701 tgtaacatgtatcatttgcatttttagAATTTCTGGCCCAAAACAGGATAGTGCATAGGATCTGGCTGCCCGAATGTTCTTAATCTCCGATAGATTCGCA
3801 GCATCAAGATAGCAGATTTTGGCTgggtatctctgaaagttcaatgattagtatatgtgtttttttttaagCTGAGTGCAGATGTGTATCATGA
3901 GAACGCTGACCGAAAGTCCGGAGGAAGTGGCAAGCTGCCCATCAAGTGGCTCGCGTGGAGTCCCTACCCACAGGTTGTACACAGTCAAGGACGTgtg
4001 taagtatatatttttggtaagtccgaagcagtttgggtgatattctaccctcttagTTGGTCTTTGGTGTGCTGCTCTATGAGATCACCACCTTCG
4101 GTGGAATGCCATATCCGTCGGTGTCTCCAGTGATCTCTTGCAGCTACTGGCACAAGGCTCATGGATGAAGCGACCGGAGGATGTACGCAAGAAATgtg
4201 ggtatcagtgcttagcggaagtcataatttggtagcatttgcattcagcagGTTTCCCTGATGGAAGAGTCTGGAGCTCCGTGCCATCAGACGCG
4301 CAACATTTTCCGCCCTTAAACACAGACTGGTGGCATGATTTTGGCCACTAAGCATGTTCGAAAGGCTGAACAACTGCAAGCTGCAACCGAGTCAAA
4401 PTTFSALKHRLGGMILATNDVTPERLKLQATATESK
4501 ATTAAGCTCATGTACGGTCTAAACAGtagtaattctttaaagacaattgtatacgttactttcttaccttttacaatttagTAAAGTGGAGCAAGT
4601 LKSCDGLNS
4701 GCCATCGGGAAGAGCTATACCTAGAACCTTTGAATTAATGATCTGCTTCAAGATTATAATGAACGAGTGCATAACATCTTAATTCGAGTTCCAAT
4801 PC E E E L Y L E P L N - -
4901 TCAGACAATGCTATGCCGACGATACCTTGATCATGaatataaactaaagctagcctaagaaaataaattttcttttataagaaaataacaaatgat
5001 ttatagaataaactcttcagcagtcgcttttatctctcagaataa

FIG. 4 Genomic and cDNA nucleotide sequence of the *torso* gene and the predicted *torso* protein sequence. The nucleotide sequence of the cDNA clone pCD7 is in upper case. Introns and genomic sequences not represented in the cDNA clone are in lower case. The predicted amino-acid sequence is shown below the nucleotide sequence, commencing at nucleotide (nt) 897. Amino-acid residues are numbered in italics. A putative CAAT-(nt 518) and TATA box (nt 570), the conserved heptanucleotide at the presumptive transcription start site corresponding to the consensus ATCA(G/T)T(C/T) (ref. 12) (nt 597), the in-frame stop codons (nt 726, nt 4,537, nt 4,540) and the AATAAA polyadenylation signal (nt 4,636) are underlined. The putative transmembrane sequence is doubly underlined. Possible N-glycosylation sites³⁷ (consensus: N-X-(Ser/Thr)-X, with N as the glycosylation site and X any amino acid except Pro) in the amino-terminal half of the protein sequence are in bold face. The P-insertion site of the *tor*^{TC17} mutation is between nt 944 and 945.

METHODS. Sequences were determined by dideoxy sequencing³⁸ using Sequenase (United States Biochemical Corporation) according to the manufacturer's instructions. Two strategies were used to prepare single-stranded DNA templates for sequencing. A genomic *Xba*I (nt 1411)–*Sac*I (nt 4283) fragment was randomly sheared by sonication and cloned into M13mp10. The rest of the genomic fragment and the cDNA was sequenced by cloning defined restriction fragments into M13mp18 or M13mp19 vectors. The complete sequence of both strands was determined for the genomic sequence. Three bases differ between the genomic sequence and the cDNA clone, probably as a result of reverse transcriptase errors during the construction of the cDNA library. In the cDNA, nt 707 is G, nt 2,320 is T and 2,568 is G. The resulting amino-acid change is Ile (405) to Val. We used the amino-acid sequence derived from the genomic sequence for further analysis. Sequence analysis was carried out using PCGENE software package (GENOFIT, Switzerland).

involved in the phosphotransfer reaction to tyrosine²⁰. The homologies between the *torso* tyrosine kinase domain and the catalytic domain of other tyrosine kinases range from 42.4% for *ret* (ref. 21) to 29.9% for *eph* (ref. 22). This overall conservation between the *torso* peptide and tyrosine kinases derived from evolutionarily distant organisms such as mammals, strongly suggests that the *torso* protein functions as a protein kinase.

In addition to amino-acid identity within the tyrosine kinase domain, the *torso* sequence shares structural features with the subclass of receptor tyrosine kinases (RTKs). These molecules contain a relatively large extracellular domain, a single transmembrane domain, a juxtamembrane domain of 41–50 amino acids adjacent to the tyrosine kinase domain and a carboxy-terminal tail of variable length (reviewed in ref. 23). Because all these features are also present in the *torso* protein, it is tempting to speculate that *torso* encodes a receptor tyrosine kinase. As in three other RTKs, the platelet-derived growth-factor receptor (PDGF-R), macrophage growth factor receptor (CSF-1-R), and the putative receptor *c-kit*, which have been classified as type-III RTKs (ref. 23), the catalytic domain of the *torso* protein is interrupted by a stretch of hydrophilic amino acids. The class III RTKs are further characterized by an immunoglobulin-like structure in the extracellular domain which includes cysteines and conserved surrounding residues²³. This immunoglobulin-like structure is absent in *torso* and comparison of the extracellular domains does not reveal similarities to other RTKs. Indeed, the amino-terminal half of the *torso* polypeptide shows no significant homology with any published sequence of the SWISS-PROT 6.0 and NBRF Protein 16.0 data-bases: *torso* therefore encodes a new type of receptor tyrosine kinase.

Conclusions

The proposed structure of the *torso* protein and its similarity with growth factor RTKs suggest that the *torso* peptide is a transmembrane protein with an extracytoplasmic domain acting as a receptor and a cytoplasmic domain containing tyrosine kinase activity. RTKs are normally activated by the binding of a ligand to the extracytoplasmic receptor, resulting in the phosphorylation of specific proteins and eventually producing a pleiotropic response, including proliferation and differentiation²⁴. We propose that *torso* participates in such a signal transduction mechanism, and that the generation of the signal and its subsequent transmission from the membrane-associated *torso* product to zygotic target genes are functions of the other terminal-class genes. Another example of a signal-transduction

process involved in pattern formation may occur in the developing eye of *Drosophila*; the product of the *sevenless* gene, which is required for the determination of the R7 photoreceptor cell, has also been identified as a putative RTK (reviewed in ref. 25).

Although genetic evidence indicates that *torso* is normally activated only at the poles⁷, cytoplasmic transplantation studies⁷ and the homogeneous distribution of the *torso* mRNA suggest that the *torso* product itself is not localized. Instead, the local activation of *torso* may be achieved through a spatially restricted ligand molecule. By analogy with other RTKs which reside in the cell membrane, the *torso* product is probably incorporated into the cytoplasmic membrane of the oocyte and early embryo. Its extracellular domain would then be in the perivitelline space, the region between the oocyte cytoplasmic membrane (oolemma) and the vitelline membrane surrounding the oocyte. If so, the *torso* protein could serve as a receptor for a ligand molecule which is present in a spatially restricted fashion in the perivitelline space. Such a ligand molecule could reach the perivitelline space by secretion either from the oocyte or from the somatic follicle cells which surround the oocyte during oogenesis. In this respect it is notable that there are specialized follicle cells at the anterior and posterior end of the follicle²⁶, suggesting the possibility that a subpopulation of follicle cells may be responsible for producing the spatially restricted molecule necessary for the activation of *torso*. A potential candidate for encoding such a ligand is *torso-like*, which is distinct from the other *torso*-group genes in that it is dependent upon the somatic rather than the germ-line cells of the ovary (H.-G. Frohnhofer, personal communication). A precedence for transfer of information from the somatic follicle cells to the oocyte is found in the establishment of the dorso-ventral axis in *Drosophila*. In this system, a mutation in the soma-dependent gene *torpedo* results in a ventralization of the embryo²⁷.

The identification of *torso* as an RTK also suggests a molecular explanation for the *torso* gain-of-function phenotype. Because it is likely that the *torso* protein is present all along the anteroposterior axis of the embryo, mutations which cause the kinase activity to become independent of ligand binding could create an ectopically active *torso* product and thereby produce a gain-of-function phenotype. Consistent with this interpretation, mutations that result in constitutively active tyrosine kinases have been isolated in other RTKs²³. The dorsal-group gene *Toll* also exhibits both gain- and loss-of-function alleles that produce opposing phenotype²⁸, and like *torso*, *Toll* encodes a transmembrane protein²⁹ which is believed to be activated in a spatially restricted manner³⁰. Although the biochemical activity of *Toll*

FIG. 5 Hydropathy analysis and structural organization of the predicted *torso* protein. *a*, Hydropathy analysis. The 923 amino-acid long *torso* protein sequence was scanned using the program of Kyte and Doolittle¹⁶. Hydrophobicity results in positive and hydrophilicity in negative values. *b*, Structural organization of *torso* protein. Landmarks in the sequence are indicated schematically: signal sequence (ss) and transmembrane domain (TM) are cross-hatched, the tyrosine kinase domains (according to Hanks³⁹) are hatched.

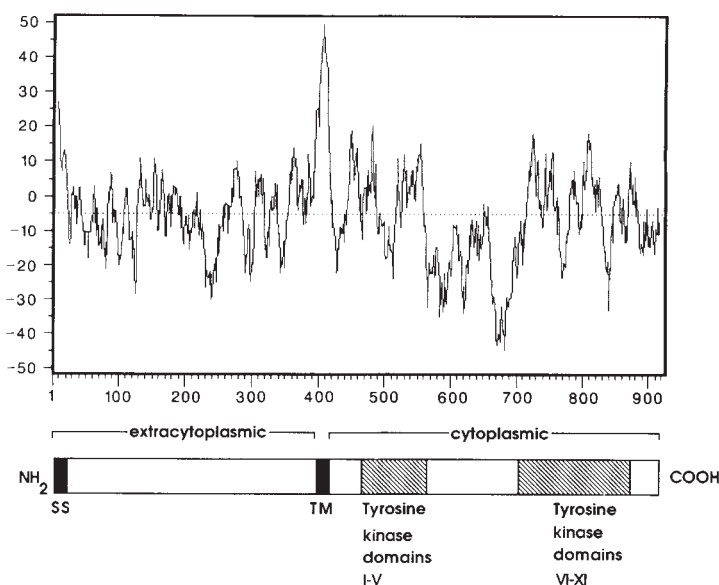


FIG. 6 Comparison of the putative tyrosine kinase domain of the *torso* gene product with the corresponding domains of the products of the *ret* (ref. 21), human *c-src* (ref. 40), *Drosophila* EGF-receptor homologue (DER, ref. 41) and platelet-derived growth-factor receptor (PDGFR; ref. 42) genes. Residues identical to the *torso* protein are in bold face and underlined. Classification into subdomains is according to Hanks *et al.*³⁹. The lysine residue involved in the phosphotransfer reaction is marked by an arrow. Two sequence patterns which are thought⁴³ to distinguish all protein kinases from all other known proteins are marked with asterisks. The consensus pattern that specifies amino-acid specificity of the kinase to tyrosine residues, DLAARN, (in the catalytic domain VI) (ref. 39) is marked by #.

subdomain	----- I -----	----- II -----	----- III -----	----- IV -----
<i>tor</i>	473 HSVLLD VLGEGAFGLVRRG YVKK-----RO VAVKLLKDEPNDD EVYAFK CTQMLKAVGKHPN IVGIVGVSTFRFS NOMML			
<i>ret</i>	455 KNLVLG KTLEGEFGK VVKATAFHLKGRAGYTT VAVMLKENASPS ELRDLL SEFNVLKQV ---N HPHVKLYGAC SQD-GPL LL			
<i>src</i>	265 ESLR EVK LGOCFCFCFVW MGTWNG-----T TRV AI IKTLK PGTM---S PEAF LQ BAQVMKL ---R HEKLV QYAVVSE---EPI YI			
DER	685 AELRKGG VLGMAFG RVYK GVV PEGEN-VKIP VAIKEL LKSTGAESSE EF L RAY IMASE-E HVN LLKLLAVCMS---S QMM			
PDGFR	565 DQLVL GR T LGSCAFG OVVEATAGLSHSQATMK VAVKMLK STARSS EKA LM SEKIM SH CPH IN VN VL LG ACTKG-GPI YI			
subdomain	----- V -----			
<i>tor</i>	549 LI BYCS LGSL QNF LR EW KFRQEQNAIG LKK N LEQ NVD NRR FNRLPR NS IHDRIEDINN SML STVE ES ESDQTHSSRCETY T			
<i>ret</i>	536 I VEY AKY GSLR GL FLR SRKVGPGYLG SG SR NS SSLDHPDERA-----			
<i>src</i>	337 VTYMS K GSLLD FL K GETGKY-----			
DER	764 ITQL MP L GL LDYVR N RNDK-----			
PDGFR	646 I TEY CRY GD LV Y L HR NKHTFLQRH SN KHC PP SAEL YS N AL PVG FS LP SH LNLTGESDGGYMDMSK DE SIDYV P MLDMKGDI K			
<i>tor</i>	632 LTRITNAAD NG KY G LEDIENIGGS Y IPKTA E APKDQ PK RKLKPQ PK KDSKQ DF SKDN KK RIFENKEY FD CLDSSDT K PRIP LK			
<i>ret</i>	579 -----			LT
<i>src</i>	358 -----			LR
DER	784 -----			IG
PDGFR	729 YADIESPSY MAP DN Y VP S APERT Y ATRLINDSPV-----			LS
subdomain	----- VI -----	----- VII -----	----- VIII -----	----- IX -----
<i>tor</i>	715 YAD LLD IA Q QV AG ME FL AQ N KV V HRD LAARN VL ISVDR SI K IA D FGL SRD YV EN YR KSG SG K LPI K W LA ES LTHQ VYT			
<i>ret</i>	581 MGD LIS FA W Q IS Q MOY LA E ML V HRD LAARN IL VAEG R K MT IS DF GLSRD YV ED S V KR ---S Q GRIP V K W LA ES LFDH YIT			
<i>src</i>	360 LPOL VD MA Q IAS G MAYVER MN Y VRD LAARN IL VGEN LV C VA D FGL AR LD NE Y TA R ---G GA K FP IK TA PE AA LYGR FT			
DER	786 SKA IL N W ST Q IA K MS Y LE E K RI V HRD LAARN VL VR LL ---A GE D H D FGL AK LL SSDS NE Y KA ---A G K MP IK W LA ES L CI R N RV FT			
PDGFR	766 YTD LV GS Y Q VANG MD FL A SK N V HRD LAARN VL ICE GL V K IC DF GL ARD IMRDS NY ISK --- G ST Y LP L K W MA PE S IF NS LY T			
subdomain	----- X -----	----- XI -----		
<i>tor</i>	798 QS SD VNS FGV LL WE IT TL GG MP Y SV SP SD LL Q LL RO GH RM K PE GC TO EM FS LM ES CS W SS VP SH PT FS AL K HL GG ML AT			
<i>ret</i>	663 TS SD VNS FGV LL WE IT TL GG NP Y GP IP PER LF N LL KT GH RM K PE PD NC SE EM Y RL MC Q W K Q EP DK RP VE AD IS K LE K MM V KR			
<i>src</i>	441 IK SD VNS FG LL TE LT TL GR VP Y GM VN REV LD Q VE R GY MP CP EP CS EL HD MC Q W K PE EP EP TF E Y L Q AF LE D Y FT ST			
DER	867 SK SD VNS FGV TI WE LT TL GR PHEN IP AK DP IP DL EV GL KE LP EP IC SLDI Y CT LL SC WH LD AA MR PT F K OL TT VF AE F ARD P			
PDGFR	848 TL SD VNS FG LL WE IT TL GG TP Y EP PM NDQ Y NA IK RG Y MA Q PA HS DE IV E IK Q K W E EP ET RP PF S OL V LL ER LL GE G			

is not yet known, its similarities to *torso* support the hypothesis²⁹ that it functions as a receptor molecule.

The information generated by binding of the putative ligand molecule must ultimately be interpreted by the terminal zygotic genes. One maternally expressed gene which might be involved in carrying the signal downstream of *torso* is *l(1)polehole*. This gene has both maternal and zygotic functions and germ-line mosaic analysis⁵ has shown that an embryo devoid of maternal *l(1)ph* mRNA but fertilized with a wild-type sperm develops a *torso* phenotype. The product of *l(1)ph* is likely to be identical to that of *l(1)raf* (refs. 31, 32), a homologue of the vertebrate serine/threonine kinase *Raf-1* (ref. 33). In addition, genetic evidence suggests that *l(1)ph* acts downstream of *torso* (M. Klingler, personal communication). Thus, signal transduction in the determination of terminal structure might involve a kinase cascade mechanism.

In summary, we propose that the *torso* gene encodes a receptor tyrosine kinase which is incorporated into the oolemma, but is not localized to the terminal regions. Its spatially restricted activity may be due to a local activation of the tyrosine kinase at the anterior and posterior ends of the embryo by binding of a localized ligand molecule to the extracellular domain of the *torso* protein. (In *torso* gain-of-function mutations, however, *torso* activity would be independent of ligand binding and the tyrosine kinase active everywhere.) Activation of *torso* would result in enhanced tyrosine phosphorylation of target proteins and the original signal would then be amplified by a cascade mechanism, in which at least one other kinases is involved. The activation of *torso* would ultimately lead to the regionalized expression of zygotic target genes, the next hierarchical level in the development of terminal structures of the *Drosophila* embryo. □

Received 28 December 1988; accepted 22 February 1989.

- Nüsslein-Volhard, C., Frohnhöfer, H. G. & Lehmann, R. *Science* **238**, 1675-1681 (1987).
- Schüpbach, T. & Wieschaus, E. *Wilhelm Roux Arch. dev. Biol.* **195**, 302-317 (1986).
- Degelman, A., Hardy, P. A., Perrimon, N. & Mahowald, A. P. *Dev. Biol.* **115**, 479-489 (1986).
- Perrimon, N., Mohler, D., Engstrom, L. & Mahowald, A. P. *Genetics* **113**, 695-712 (1986).
- Perrimon, N., Engstrom, L. & Mahowald, A. P. *Dev. Biol.* **110**, 480-491 (1985).
- Strecker, T. R., Merriam, J. R. & Lengyel, J. A. *Development* **102**, 721-734 (1988).
- Klingler, M., Erdelyi, M., Szabad, J. & Nüsslein-Volhard, C. *Nature* **335**, 275-277 (1988).
- Seear, L. L., Jörker, R. S., Bingham, P., Voelker, R. A. & Greenleaf, A. L. *Cell* **31**, 585-592 (1982).
- Goldberg, D. A., Posakony, J. W. & Maniatis, T. *Cell* **34**, 59-73 (1983).
- Frigerio, G., Burri, M., Bopp, D., Baumgartner, S. & Noll, M. *Cell* **47**, 735-746 (1986).
- Kobayashi, S., Mizuno, H. & Okada, M. *Dev. Growth Differ.* **30**, 251-260 (1988).
- Hunt, D., Klemenz, R. & Gehring, W. J. *Cell* **44**, 429-438 (1986).
- Breathnach, R. & Chambon, P. A. *Rev. Biochem.* **50**, 349-383 (1981).
- Fitzgerald, M. & Shenk, T. *Cell* **24**, 251-260 (1981).
- Cavener, D. R. *Nucleic Acids Res.* **15**, 1353-1361 (1987).
- Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105-132 (1982).
- Klein, P., Kanehisa, M. & Delisi, C. *Biochim. biophys. Acta* **815**, 468-476 (1985).
- Heijne, G. V. *J. molec. Biol.* **184**, 99-105 (1985).
- Heijne, G. V. *Nucleic Acids Res.* **14**, 4683-4690 (1986).
- Kamps, M. P. & Sefton, B. M. *Molec. cell. Biol.* **6**, 751-757 (1986).
- Takahashi, M. & Cooper, G. M. *Molec. cell. Biol.* **7**, 1378-1385 (1987).
- Hirai, H., Maru, Y., Hagiwara, K., Nishida, J. & Takaku, F. *Science* **238**, 1717-1720 (1987).
- Yarden, Y. & Ullrich, A. A. *Rev. Biochem.* **57**, 443-478 (1988).
- Hunter, T. & Cooper, J. A. A. *Rev. Biochem.* **54**, 897-930 (1985).
- Basler, K. & Hafen, E. *Trends Genetics* **4**, 74-79 (1988).

- Brower, D. L., Smith, R. J. & Wilcox, M. *Nature* **285**, 403-405 (1980).
- Schüpbach, T. *Cell* **49**, 699-707 (1987).
- Anderson, K. V., Jürgens, G. & Nüsslein-Volhard, C. *Cell* **42**, 779-789 (1985).
- Hashimoto, C., Hudson, K. L. & Anderson, K. V. *Cell* **52**, 269-279 (1988).
- Anderson, K. V., Bokla, L. & Nüsslein-Volhard, C. *Cell* **42**, 791-798 (1985).
- Nishida, Y. *et al. EMBO J.* **7**, 775-781 (1988).
- Mark, G. E., MacIntyre, R. J., Digan, M. E., Ambrosio, L. & Perrimon, N. *Molec. cell. Biol.* **7**, 2134-2140 (1987).
- Bonner, T. I. *et al. Nucleic Acids Res.* **14**, 1009-1015 (1986).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Ingham, P. W., Howard, K. R. & Ish-Horowitz, D. *Nature* **318**, 439-445 (1985).
- King, R. C. *Ovarian Development in Drosophila melanogaster* (Academic, New York, 1970).
- Bause, E. *Biochem. J.* **209**, 331-336 (1983).
- Sanger, F., Nicklen, S. & Coulson, R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
- Hanks, S. K., Quinn, A. M. & Hunter, T. *Science* **241**, 42-52 (1988).
- Anderson, S. K., Gibbs, C. P., Tanaka, H.-J. K. & Fujita, D. *J. molec. cell. Biol.* **5**, 1122 (1985).
- Livneh, E., Glazer, L., Segal, D., Schlesinger, J. & Shilo, B.-Z. *Cell* **40**, 599-607 (1985).
- Yarden, Y. *et al. Nature* **323**, 226-232 (1986).
- Baird, A. & Claverie, J. M. *Nature* **331**, 22 (1988).

ACKNOWLEDGEMENTS. We thank T. Schüpbach for the gift of the *tor*^{TC17} stock, H. Jäckle for the Oregon P2 library, M. Noll for the cDNA library, P. Ingham for advice, our colleagues in Tübingen for discussions, interest and comments on the manuscript and R. Grönke-Lutz for photographs. This work was supported by the Leibnitz program of the Deutsche Forschungsgemeinschaft. F.S. is a fellow of the Boehringer Ingelheim Fonds.

Production of self-absorbed synchrotron spectra steeper than $\nu^{5/2}$

Martijn de Kool & Mitchell C. Begelman

Joint Institute for Laboratory Astrophysics, University of Colorado and National Institute of Standards and Technology, Boulder, Colorado 80309-0440, USA

SELF-ABSORBED synchrotron radiation produced by electrons with a power-law distribution of energies has a unique spectral shape: intensity $I_\nu \propto \nu^{5/2}$ (where ν is frequency), irrespective of the power law index of the electrons¹. This well-known result has been applied recently to the 'far-infrared turnovers' observed in the spectra of many radio-quiet active galactic nuclei (AGNs)²⁻⁴. It has been asserted that the measurement of a spectral index greater than 5/2 at frequencies below the turnover is incompatible with the physics of self-absorbed synchrotron sources. Several observations suggesting larger indices (steeper slopes)⁵⁻⁷ between the far-infrared and millimetre bands have been interpreted as evidence that emission by warm dust, not synchrotron radiation, dominates the far-infrared spectrum. Rees⁸ has, however, pointed out that this assertion is not necessarily true. Here we show that plausible electron energy distributions can lead to self-absorbed synchrotron spectra which are steeper than $\nu^{5/2}$ over 1-1.5 orders of magnitude in frequency. This indicates that none of the existing observations are in fact incompatible with self-absorbed synchrotron radiation as the source.

Consider an electron energy distribution consisting of the sum of two power laws,

$$N_\gamma d\gamma = C_1 \gamma^{-p_1} d\gamma + C_2 \gamma^{-p_2} d\gamma \quad (1)$$

where γ is the electron Lorentz factor and C_1 and C_2 are constants. Without loss of generality we shall assume in the following that $p_1 > p_2$. As $\gamma > 1$, this implies that the steeper power law can become of importance only if $C_1 > C_2$. The synchrotron intensity in the self-absorbed limit is given by the ratio of the emission coefficient to the absorption coefficient, which for $p_1, p_2 > \frac{1}{3}$ reduces to

$$I_\nu = \frac{j_\nu}{\kappa_\nu} \equiv \frac{C_1 j_1 \nu^{-(p_1-1)/2} + C_2 j_2 \nu^{-(p_2-1)/2}}{C_1 \kappa_1 \nu^{-(p_1+4)/2} + C_2 \kappa_2 \nu^{-(p_2+4)/2}} \quad (2)$$

where the analytic expressions for j and κ are well-known (see, for example, ref. 1). The coefficients j_1, κ_1 and j_2, κ_2 depend

on p_1 and p_2 respectively, and on the cyclotron frequency ν_B . Equation (2) assumes the ultra-relativistic approximation for synchrotron emission. As the effects that we are considering are likely to be caused by relativistic electrons with $\gamma \geq 10$ (see below), this approximation is justified. For simplicity, we also neglect effects of polarization and pitch-angle distribution. A proper treatment of these effects⁹ yields results that differ from our estimate by less than a few per cent. Defining $\eta \equiv C_2 j_2 / C_1 j_1$, $\chi \equiv C_2 \kappa_2 / C_1 \kappa_1$ and $q \equiv (p_1 - p_2)/2$, we find that the slope of the spectrum is given by

$$\alpha \equiv \frac{d \log I_\nu}{d \log \nu} = \frac{5}{2} + \frac{q \nu^q (\eta - \chi)}{(1 + \eta \nu^q)(1 + \chi \nu^q)} \quad (3)$$

Evaluating η and χ using the standard expressions for power-law synchrotron emission and absorption, it can easily be shown that $\alpha > \frac{5}{2}$ for all ν , although the deviation from $\frac{5}{2}$ becomes arbitrarily small in the limits of both large and small ν . Differentiating equation (3) with respect to ν yields the value ν_m at which the maximum slope α_m occurs. The latter is given by

$$\alpha_m = \frac{5}{2} + \frac{q[(\eta/\chi)^{1/2} - (\chi/\eta)^{1/2}]}{[1 + (\eta/\chi)^{1/2}][1 + (\chi/\eta)^{1/2}]} \quad (4)$$

This maximum slope depends only on p_1 and p_2 . In Fig. 1 we show a contour plot of α_m as a function of p_1 and p_2 , from which we see that a significant deviation from $\frac{5}{2}$ occurs if $p_1 - p_2 > 2$. To illustrate the width of the interval in frequency that is affected by the steepening, Fig. 2 shows a contour plot of the spectral index α in the (ν, p_1) plane, for $p_2 = 3$. (The latter value is thought to apply in AGNs, in which an optically thin synchrotron spectrum proportional to ν^{-1} is usually observed.) In Fig. 2 the frequency is normalized to ν_m , so that the result is independent of the actual values of C_1 and C_2 . The spectral index is significantly steeper than $\frac{5}{2}$ over 1-1.5 decades in frequency.

Our numerical experiments for arbitrary electron distributions indicate that the main feature of the electron distribution responsible for a spectrum steeper than $\nu^{5/2}$ is

$$\frac{d^2 \log N_\gamma}{d \log^2 \gamma} > 0 \quad (5)$$

Although we cannot prove this condition rigorously, it can be understood by considering the case of two power laws. For a power-law electron distribution, a photon with a given frequency is on average absorbed by an electron with an energy lower than the average energy of an electron that emits at this frequency. (See, for example, ref. 1, equation [6.52].) This means that the

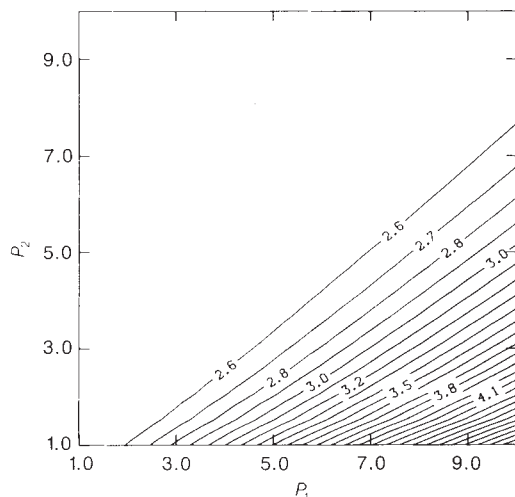


FIG. 1 Contour plot of the maximum spectral slope α_m as a function of the power-law indices p_1 and p_2 .

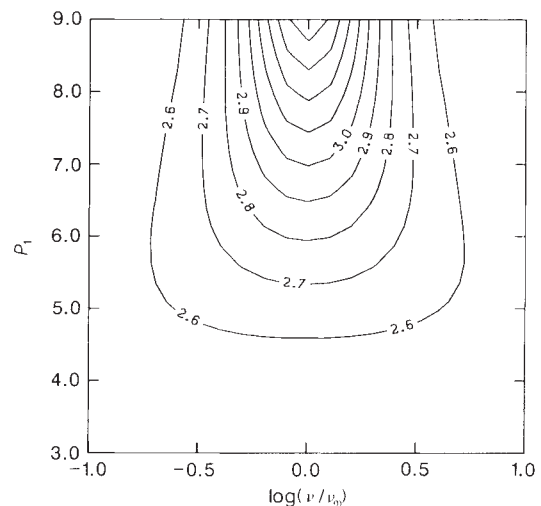


FIG. 2 Contour plot of the spectral slope as a function of frequency and p_1 , for a fixed value of $p_2 = 3$. The frequency is normalized to ν_m , at which the maximum α_m occurs.

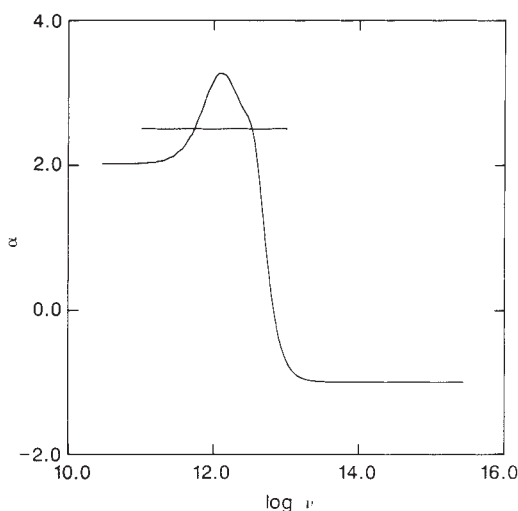


FIG. 3 The spectral slope as a function of frequency for a self-absorbed synchrotron source containing a distribution of relativistic electrons that is a combination of a power law and a relativistic maxwellian (equation (6)). At low frequencies the spectrum is thermal ($\alpha = 2$); it steepens considerably before becoming optically thin ($\alpha = -1$) at high frequencies. The illustrated spectrum is for $\beta = 1$ and $C_1/C_2 = 1$. The horizontal line represents a spectral slope of $\frac{5}{2}$.

absorption coefficient in equation (2) (the denominator) switches to being dominated by the first term (power law 1) at a higher frequency than the emission term (the numerator), so that the spectral index tends to $\frac{5}{2} + q$ rather than $\frac{5}{2}$. Thus a distribution in which the number of low-energy electrons is over-represented relative to a power law extrapolation of the high-energy distribution is likely to produce a spectrum steeper than $\nu^{5/2}$ over some frequency range. Note that neither the distribution function nor the derivative of its slope need be monotonic.

How can a relativistic electron distribution with the properties necessary for α to be greater than $\frac{5}{2}$ be produced in an AGN? A significant steepening of the electron distribution at low energies, relative to a power law, occurs if (1) there is extra injection of relativistic electrons at low energies described by a steep power law, for example from Fermi acceleration produced by many weak shocks; (2) there is a tail of a relativistic maxwellian distribution connecting with the power law at low energies, a situation that arises naturally if the effect of synchrotron self-absorption on the relativistic electron distribution is taken into account^{10,11}; or (3) the net cooling rate of the relativistic electrons varies with energy in such a way that condition (5) is fulfilled. This would require that the cooling rate decrease towards lower energies more steeply than can be described by a single power law, or that there is a cooling mechanism that acts only in a restricted energy range.

The third possibility seems rather unlikely, but the first two are physically quite reasonable. Case (2), the combination of a relativistic maxwellian and a power law, is difficult to solve analytically, but we show in Fig. 3 our numerical results for the spectral index α as a function of frequency, for N_γ of the form

$$N_\gamma = C_1 \gamma^2 e^{-\beta\gamma} + C_2 \gamma^{-3} \quad (6)$$

where β is an arbitrary constant. The strong inflection point in the electron distribution at the transition between the exponential tail and the power law causes a sharp peak in α , with a width of about a decade. For $\beta \approx 1$ and a wide range in C_1/C_2 , the inflection point in the distribution (6), at which the two terms become equal, occurs for $10 < \gamma < 50$. If the usual order-of-magnitude estimate of the magnetic field strength in an AGN of 10^3 gauss is correct, this range in γ corresponds precisely to the range in energy containing the electrons responsible for the synchrotron radiation just below the turnover¹⁰.

Before comparing with observations, we must mention that there are effects that could flatten the observed spectrum from the ideal self-absorbed synchrotron spectrum we have discussed above. Prominent among these are radiative-transfer effects (if the source does not have a very sharp edge) or inhomogeneity (if parameters vary over the source surface).

The lower limits on the spectral slope below the turnover in observed AGN spectra are derived⁶ mostly from the 100- μ m and 1.3-mm fluxes. To compare this with our results we have calculated the maximum average α obtainable over such a factor-of-13 range in frequency. We find that this maximum is rather independent of the details of the model, and is about 2.75–2.8. The high value of α of 3.06 reported⁷ for wavelengths between 155 and 438 μ m in NGC4151 can also be reconciled with our models because, as Figs 2 and 3 indicate, steeper slopes can be obtained over a smaller range in frequency. These results imply that there are at present no measured spectral slopes below the turnover for which synchrotron radiation cannot be the main emission mechanism. $\square$

Received 5 December 1988; accepted 7 February 1989.

1. Rybicki, G. B. & Lightman, A. P. *Radiative Processes in Astrophysics* (Wiley, New York, 1979).
2. Edelson, R. A. *Astrophys. J.* **309**, L69–L72 (1986).
3. Edelson, R. A. & Malkan, M. A. *Astrophys. J.* **308**, 59–77 (1986).
4. Edelson, R. A., Malkan, M. A. & Rieke, G. H. *Astrophys. J.* **321**, 233–250 (1987).
5. Engargiola, G., Harper, D. A., Elvis, M. & Willner, S. P. *Astrophys. J.* **332**, L19–L22 (1988).
6. Chini, R., Kreysa, E. & Biermann, P. L. *Astr. Astrophys.* (submitted).
7. Edelson, R. A., Gear, W. K. P., Malkan, M. A. & Robson, E. I. *Nature* **336**, 749–751 (1988).
8. Rees, M. J. *Mon. Not. R. astr. Soc.* **136**, 279–291 (1967).
9. Melrose, D. B. *Plasma Astrophysics Vol. 1*, (Gordon and Breach, New York, 1978).
10. De Kool, M., Begelman, M. C. & Sikora, M. *Astrophys. J.* **337**, 66–77 (1989).
11. Ghisellini, G., Guilbert, P. W. & Svensson, R. *Astrophys. J.* **334**, L5–L8 (1988).

ACKNOWLEDGEMENTS. We thank S. Phinney for comments on an earlier version of this paper. This work was supported in part by the NSF, NASA and grants from Rockwell International Corporation and the Alfred P. Sloan Foundation.

Anisotropic optical and X-ray emission in quasars

N. Jackson, I. W. A. Browne, D. W. Murphy & D. J. Saikia

Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire SK11 9DL, UK

IT is of fundamental importance in the study of quasars to understand whether or not their continuum emission is isotropic, because this affects the overall energy budget and can also help to distinguish between the different models proposed for their central regions¹. The reported small range of emission-line equivalent widths in quasars have earlier been used to argue against a relativistically beamed or anisotropic continuum^{2,3}. Here we use recent spectroscopic observations of 46 radio-loud quasars to show that the equivalent width of the [O III] 5,007-Å emission line is strongly anticorrelated with the degree of radio core prominence R , which has been found to be a statistical indicator of orientation relative to the line of sight^{4–6}. This observed anticorrelation shows that the quasar continuum is anisotropic and provides important evidence in favour of relativistic-beaming unified schemes^{4,7,8}. We also show that the strength of the [O III] emission line is independent of R , consistent with unified schemes.

In relativistic beaming models, the radio emission from the core can be strongly Doppler boosted at small angles to the line of sight, because the material is believed to be moving relativistically⁸. On the other hand, the extended radio emission is at most mildly relativistic. Thus in such models the ratio R of core emission to extended radio emission is a useful indicator of source orientation, with R increasing as the angle to the line of sight decreases⁴. Several statistical studies have shown the radio properties of quasars to be consistent with such a scheme^{4,5,9,10}.

A principal argument against extending such a scheme to include relativistic beaming of the optical and X-ray continuum¹¹ derives from the small range of emission-line equivalent width in quasars^{2,3}. As the line luminosity should not depend on orientation, relativistic beaming of the continuum should be reflected in a spread in equivalent width, with the high- R objects having systematically lower equivalent widths than low- R objects.

To investigate this problem using a homogeneous set of spectra, we observed 46 radio-loud, low-redshift quasars with the Faint Object Spectrograph¹² on the Isaac Newton Telescope. Because we wished to study relativistic beaming of both the optical and X-ray continuum, most of the objects were selected on the grounds of having been studied at X-ray energies (2 keV) by the Einstein Observatory. Nearly all such objects with $z \leq 0.8$ were observed, and several other quasars were added to fill gaps in the observing schedule, usually on the grounds of ease of observation and membership of the 3C catalogue. We emphasize that no prior selection by emission line strength has been imposed other than that implied by the necessity that all objects have measured redshifts and are classified as quasars by Véron and Véron¹³.

The spectral resolution of these observations is about 18 Å and the wavelength coverage is 5,000–9,500 Å. In every object, lines of H β and [O III] 5,007-Å emission are observable. We have measured [O III] in all but three objects; for these three we have upper limits to [O III]. Full details of the observations,

calibration and data reduction are given elsewhere¹⁴. We have taken X-ray and radio luminosities from previous compilations^{11,15,16}, supplemented in a few cases with radio data from our own unpublished Very Large Array (VLA) observations.

Here we focus on the [O III] line fluxes and their correlations with R , the ratio at 5 GHz of the core luminosity to the extended radio luminosity L_{ext} in the quasar's rest frame. We use [O III] because it is easier to measure than H β and is less affected by contamination with broad Fe II blends¹⁴; moreover, it is emitted further from the quasar nucleus (a few hundreds of pc rather than a few pc) and orientation-dependent obscuration effects should interfere less with its flux.

In Fig. 1a and b we plot [O III] optical and X-ray equivalent widths against R (X-ray equivalent width is defined as the ratio of [O III] line flux to the K-corrected¹¹ X-ray continuum at 2 keV). The spread in equivalent widths is at least an order of magnitude and, contrary to earlier suggestions¹⁷, there is a very significant anticorrelation between the equivalent width and R . The formal levels of significance are listed in Table 1. If we accept relativistic beaming models, this result provides strong evidence that some part of the quasar continuum emission increases when viewed at small angles to the line of sight. We have examined the possibility that the above anticorrelation could be a secondary effect of a primary correlation between equivalent width and extended luminosity together with a selection-induced anticorrelation between extended radio luminosity and R . Both a partial correlation coefficient analysis¹⁸ (Table 1) and comparisons of pairs of quasars matched in extended radio luminosity show that the equivalent width: R anticorrelation is significant. Moreover, in a virtually complete subsample (13 objects out of 17) from quasars having a flux at 2.7 GHz ≥ 1.5 Jy (ref. 19) and a redshift $z \leq 0.8$ in the RA (right ascension) ranges over which we have data, the rank correlation is -0.74 , giving a significance level of 99%. This suggests that selection effects are not responsible for the anticorrelation.

We can use our data to investigate whether the line and continuum luminosities lend independent support to the relativistic beaming unified scheme. If the low- R and high- R objects are intrinsically similar but differ only in orientation, their [O III] luminosities should be similar for a given extended radio luminosity. We indeed find that the hypothesis that the two distributions are indistinguishable cannot be rejected by a Kolmogorov-Smirnov test at the 80% level for 13 pairs of core- and lobe-dominated quasars matched in extended radio luminosity. Earlier studies have shown that, again for a given extended radio luminosity, the core-dominated (high- R) quasars have more optical and X-ray continuum than the lobe-dominated quasars^{11,20}. We find a similar result using our data. The

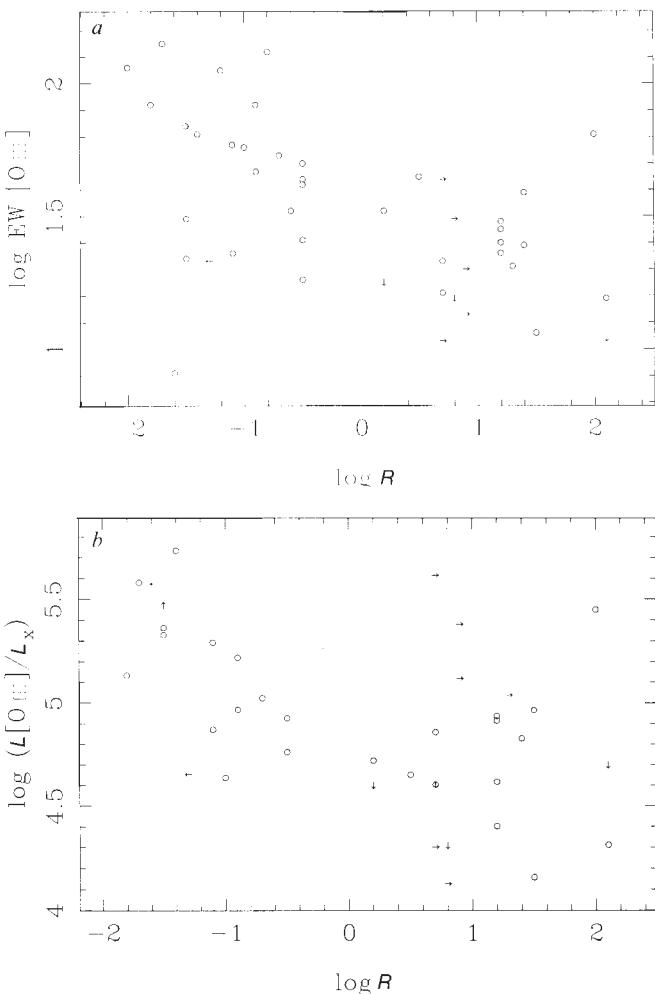


FIG. 1 a, Logarithm of optical equivalent width plotted against $\log R$ (defined in the text). Measured points are shown as circles, limits as arrows. b, Logarithm of X-ray equivalent width ([O III] luminosity divided by 2-keV X-ray luminosity L_x) plotted against $\log R$. Symbols are the same as in a.

TABLE 1 Correlations of various parameters with R

Parameter	Rank correlation	Number of points	Significance (%)
EW [O III]*	-0.53	46	99.9
EW [O III]†	-0.52	36	99.9
X-ray EW [O III]*	-0.47	38	99.5
X-ray EW [O III]†	-0.55	27	99.5

Correlations using 46 points			
Parameters	Rank correlation	Partial RC	Significance (%)
EW [O III]: R	-0.53	-0.47	99.9
EW [O III]: L_{ext}	0.29	0.04	60
$L_{\text{ext}}: R$	-0.49	-0.41	99.5

EW: equivalent width.

* Coefficients for all data, including upper and lower bounds.

† Coefficients excluding data for upper and lower bounds.

hypothesis that the distributions of continuum luminosity are identical can be rejected by the Kolmogorov-Smirnov test at the 95% level. Our conclusion is that, in addition to some isotropic emission, there is a component of quasar continuum that depends systematically on orientation.

There are at least two ways of producing directionally dependent continuum emission: by relativistic beaming of non-thermal radiation^{7,8} or by thermal radiation from an optically thick disk whose axis is parallel to the radio axis¹. These two effects dominate over different ranges of R : relativistic beaming dominates for $R \geq 1$, and disks for $R \leq 1$. Although we would argue that relativistic beaming is responsible for the extra brightness of high- R objects, sometimes leading to blazar activity, whether or not the effects of directionally dependent disk radiation are noticeable at low R remains to be investigated.

Finally we consider whether objects that do not satisfy our quasar selection criteria should be added to Fig. 1. At one extreme there are BL Lac objects, whose lines are generally too weak to allow measurement of their redshift. These objects would appear in the lower right area of Fig. 1 because they are invariably core-dominated. We strongly suspect that at least some of these objects should be included with the quasars, and this inclusion would obviously reinforce the observed anticorrelation. At the other extreme lie radio galaxies, objects with very high equivalent widths and, usually, very low R . It has been argued^{21,22} that these objects should also be considered as quasars, with their radio axes in the plane of the sky and their nuclear continuum sources and broad-line regions obscured. Although adding them to Fig. 1 would greatly increase the significance of the correlation, we feel that the case for inclusion of these objects has yet to be fully established.

We conclude that the similarity of [0111] strengths in core- and lobe-dominated quasars of the same extended radio luminosity supports models in which the only difference between the two types of quasar is viewing angle, together with the consequent Doppler beaming. The natural interpretation of our equivalent width: R anticorrelation is therefore that the optical continuum is anisotropic. This is an important result, for two reasons. First, emission models must take into account the fact that the different parts of the line-emitting region may see very different photo-ionizing continua. And second, the assumption that selection of quasars by optical brightness leads to samples with a random distribution of orientation angles²³ may not be valid. $\square$

Received 27 October 1988; accepted 16 February 1989.

- Netzer, H. *Mon. Not. R. astr. Soc.* **216**, 63-78 (1985).
- Moore, R. L. & Stockman, H. S. *Astrophys. J.* **279**, 465-484 (1984).
- Kemhavi, A., Feigelson, E. D. & Singh, K. P. *Mon. Not. R. astr. Soc.* **220**, 51-67 (1986).
- Orr, M. J. L. & Browne, I. W. A. *Mon. Not. R. astr. Soc.* **200**, 1067-1080 (1982).
- Kapahi, V. K. & Salkia, D. J. *J. Astrophys. Astr.* **3**, 465-487 (1982).
- Hough, D. & Readhead, A. C. S. *Astrophys. J.* (submitted).
- Blandford, R. D. & Rees, M. J. in *Pittsburgh Conf. on BL Lac objects* (ed. Wolfe, A.) 328-347 (Univ. of Pittsburgh, 1978).
- Blandford, R. D. & Konigl, A. *Astrophys. J.* **232**, 34-48 (1979).
- Antonucci, R. R. J. & Ulvestad, J. S. *Astrophys. J.* **294**, 158-182 (1985).
- Fugmann, W. *Astr. Astrophys.* **205**, 86-92 (1988).
- Browne, I. W. A. & Murphy, D. *Mon. Not. R. astr. Soc.* **226**, 601-627 (1987).
- Breare, J. M. *et al.* *Mon. Not. R. astr. Soc.* **227**, 909-919 (1987).
- Veron-Cetty, M. & Veron, P. *Catalogue of quasars and active galactic nuclei* 3rd edn (ESO, Garching bei München, 1987).
- Jackson, N. & Browne, I. W. A. *Mon. Not. R. astr. Soc.* **236**, 97-105 (1989).
- Hintzen, P., Ulvestad, J. & Owen, F. N. *Astr. J.* **88**, 709-758 (1983).
- Shone, D. L. & Browne, I. W. A. *Mon. Not. R. astr. Soc.* **222**, 365-372 (1986).
- Fabbiano, G., Miller, L., Trinchieri, G., Longair, M. & Elvis, M. *Astrophys. J.* **277**, 115-131 (1984).
- Macklin, J. T. *Mon. Not. R. astr. Soc.* **199**, 1119-1136 (1982).
- Peacock, J. A. & Wall, J. V. *Mon. Not. R. astr. Soc.* **194**, 331-349 (1981).
- Browne, I. W. A. & Wright, A. E. *Mon. Not. R. astr. Soc.* **213**, 97-102 (1985).
- Barthel, P. D. *Astrophys. J.* **336**, 606-611 (1989).
- Scheuer, P. A. G. in *Superluminal Radio Sources* (eds Zensus, J. A. & Pearson, T. J.) 104-113 (Cambridge University Press, 1987).
- Phinney, E. S. in *Astrophysics of Active Galaxies and Quasi-stellar Objects* (ed. Miller, J. S.) 453-496 (University Science Books, Mill Valley, California, 1985).

ACKNOWLEDGEMENTS. The INT is operated on La Palma by the Royal Greenwich Observatory at the Spanish Observatorio del Roque de los Muchachos of the Instituto de Astrofísica de Canarias. We thank S. Unger, P. Murdin and D. King for help with the observations. N.J. is grateful for a Samuel Gratrix postgraduate studentship.

Impact erosion of the primordial atmosphere of Mars

H. J. Melosh & A. M. Vickery

Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721 USA

ABUNDANT geomorphic evidence for fluvial processes on the surface of Mars suggests that during the era of heavy bombardment, Mars's atmospheric pressure was high enough for liquid water to flow on the surface. Many authors have proposed mechanisms by which Mars could have lost (or sequestered) an earlier, thicker atmosphere but none of these proposals has gained general acceptance. Here we examine the process of atmospheric erosion by impacts and show that it may account for an early episode of atmosphere loss from Mars. On the basis of this model, the primordial atmospheric pressure on Mars must have been in the vicinity of 1 bar, barring other sources or sinks of CO_2 . Current impact fluxes are too small to erode significantly the present martian atmosphere.

Although a great deal of effort has been expended on the study of the craters produced by large impacts, little work has been done on the atmospheric effects of impacts. A beginning was made by Walker¹, who showed that a projectile traversing the atmosphere of a planet could eject at most only a few times the mass of the atmosphere traversed. This conclusion generally agrees with that of Ahrens and O'Keefe², who studied the effects of the airblast produced by the impact and of the early fast ejecta on atmospheric erosion. Previously³ we used an analytical model to show that fast solid ejecta from the crater are similarly

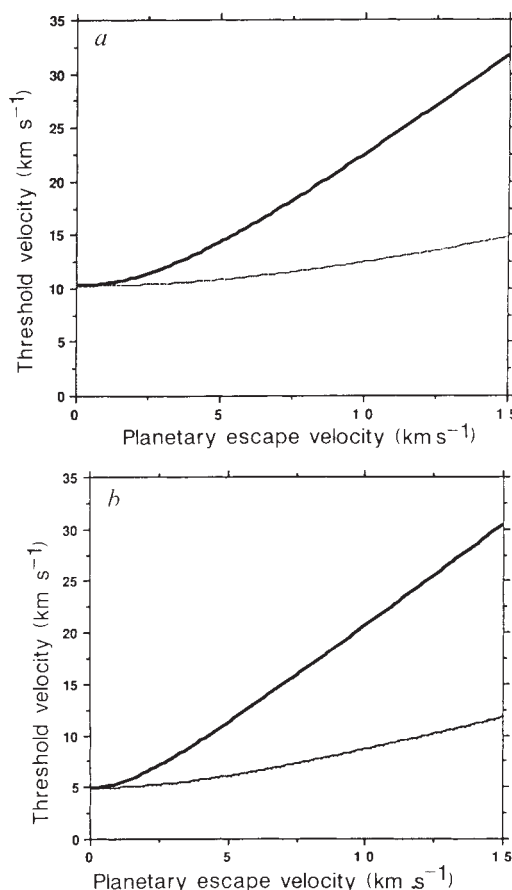


FIG. 1 Threshold impact velocity for some (light line) and most (heavy line; from equation (1) of the text) of the vapour produced in an impact to reach escape velocity of the planet on which it strikes: a, for silicate projectiles; b, for ice projectiles.

unable to eject more than a few times the atmospheric mass traversed by the projectile. However, we show here that the vapour plume created by high-speed impacts is extremely effective in ejecting atmospheric gases—for sufficiently large impacts the vapour plume may eject the entire airmass above the plane tangent to the point of impact, a result in general conformity with those of refs 4 and 5. Atmospheric erosion by impacts may therefore play an important part in the evolution of atmospheric pressure, especially on Mars because of its low gravity and small radius.

Although much of our work is based on detailed computer modelling, it is easy to construct a simple analytical model of atmospheric erosion by impacts that seems to capture most of the essentials of the process. Only a few basic equations are needed if one is willing to overlook uncertainties and subtleties that could change the results by factors of about two. The first essential feature for atmospheric erosion to occur is that the projectile must strike the planet with enough velocity for a vapour plume to form. The plume must also expand at a speed faster than the planet's escape velocity, v_{esc} . A model of vapour-plume expansion⁶ gives the mean (mass-averaged) velocity of expansion as $[2(E - H_{\text{vap}})]^{1/2}$, where E is the specific energy of the vaporized projectile and target material and H_{vap} is the vaporization energy, taken here to be 13 MJ kg⁻¹ for silicates and 3 MJ kg⁻¹ for ice. The maximum expansion velocity of the gas cloud is approximately a factor $[2\gamma/(\gamma - 1)]^{1/2}$ ($=2.82$ for $\gamma = 4/3$, where γ is the ratio of specific heats) times faster than this, so use of the mean velocity is a conservative assumption. The specific energy E is, from the Hugoniot equations, equal to $u^2/2$, where u is the particle velocity behind the shock. The maximum particle velocity u is $v/2$, where v is the impact velocity, by the planar impact approximation when both target and projectile are nearly the same material, so $E \approx v^2/8$. After a little algebra, the threshold impact velocity v_{min} for most of a vapour plume to exceed escape velocity can be shown to be

$$v_{\text{min}} = \sqrt{8 \left(\frac{v_{\text{esc}}^2}{2} + H_{\text{vap}} \right)} \quad (1)$$

Figure 1 illustrates this threshold for both silicate and icy projectiles, along with the more optimistic threshold at which some of the expanding vapour plume reaches escape velocity. We believe the more conservative limit in equation (1) is to be preferred for atmospheric-loss computations.

In the case of Mars, v_{min} is 14.3 km s⁻¹ for silicate impactors and 11.1 km s⁻¹ for icy impactors. These minima are less than the estimated r.m.s. impact velocities⁷ with either Earth-crossing asteroids (19 km s⁻¹), periodic comets (21 km s⁻¹), or parabolic comets (42 km s⁻¹). It is slightly larger than the mean impact velocity⁸ with Mars-crossing asteroids (10 km s⁻¹). Here we assume that the flux of impactors fast enough to produce a vapour plume that exceeds Mars's escape velocity is half of the total flux. During accretion, impact velocities were undoubtedly

lower so that at that time Mars could have accumulated its original atmosphere.

The second factor in estimating whether or not an impact can remove the atmosphere is that if the vapour plume is to carry away the atmosphere, the mass of vapour from projectile and surrounding vaporized target (if any) must exceed the airmass $m_{\text{ip}} \approx 2\pi P_0 HR/g$ above the plane tangent to the impact. This relation is supported by our more detailed numerical studies. Thus, we take

$$m_* \geq \frac{2\pi P_0}{g} HR \quad (2)$$

where m_* is the projectile mass just capable of removing the atmosphere above the tangent plane, P_0 is the surface atmospheric pressure, g is the acceleration of gravity, R is the radius of the planet, and H is the atmospheric scale height. In equation (2) it is assumed that the projectile and an equal mass of the target are vaporized and shocked to the same total internal energy. Although other assumptions could be made, this is one of the most conservative because it neglects a possibly significant quantity of vaporized target that may expand fast enough to reach escape velocity itself.

A potentially important effect neglected in equation (2) is the concentration of atmospheric mass at low angles from the horizon. The vapour plume expands nearly hemispherically, and thus may couple inefficiently to the atmospheric mass which is concentrated toward the horizon. Note that after the vapour plume has expanded to a few tens of the projectile diameter, adiabatic cooling condenses much of the vaporized rock into a dense, fast-moving dust cloud that follows nearly ballistic trajectories and sweeps the ambient atmosphere before it. This effect is largest for small values of H/R . Our detailed numerical computations suggest that this effect is minimal for Mars, but could be important for the Earth or Venus.

For Mars, the smallest projectile that can remove the entire airmass above the tangent plane at the present time is $\sim 4 \times 10^{13}$ kg, or a silicate object ~ 3 km in diameter. Note that equation (2) does not take account of possible enhanced vaporization in oblique impacts — such an angle-dependent phenomenon might reduce m_* by a factor of perhaps five.

These results for the mass of the projectile necessary to remove the atmosphere above the tangent plane must be supplemented with the flux of projectiles to compute the evolution of atmospheric pressure with time. The present cumulative flux (number s⁻¹ m⁻²) of projectiles with mass greater than or equal to m is parameterized by $N_{\text{cum}}(m) = am^{-b}$, where a and b are constants. Unfortunately, neither the overall flux, a , nor the slope of the distribution, b , are well-known. Values of these constants that predict the correct present lunar crater distribution via the revised Schmidt-Holsapple scaling law⁹ are $a = 1.55 \times 10^{-23}$ kg ^{b} m⁻² s⁻¹, $b = 0.47$. The slope of the crater distribution on the martian plains is the same as the lunar distribution^{10,11}, and the overall cratering rate on Mars at present is

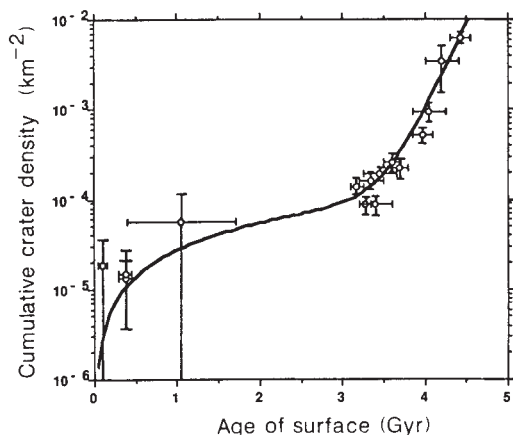


FIG. 2 Comparison between the lunar impact crater density and that predicted by the flux model of equation (5). The revised Schmidt-Holsapple scaling relations for silicate projectiles⁹ were used to equate the flux to a crater density assuming an average impact velocity of 20 km s⁻¹, mean impact angle of 45°, and a rim-to-rim diameter 25% larger than the apparent transient crater diameter. This yields a cumulative crater density for craters > 4 km diameter of $N(> 4 \text{ km}) = 2.68 \times 10^5 [7 + 4.57 \times 10^{-7} (e^{\lambda T} - 1)]$, where T is the age of the cratered surface and $\lambda = 4.53 \text{ Gyr}^{-1}$. Crater density data from the compilation in ref. 7, Table 8.4.2.

estimated to between one and four times the lunar rate, with a preferred mean of about two (ref. 7).

Using this constant cratering rate, the rate of mass loss from a planet's atmosphere dM_{atm}/dt is given by the flux $N_{\text{cum}}(m_*)$ of projectiles large enough to remove the atmosphere above the tangent plane, multiplied by the planet's surface area $4\pi R^2$, multiplied by the mass above the tangent plane $m_{\text{tp}} = M_{\text{atm}}(H/2R)$ for $R \gg H$. As m_* itself depends upon M_{atm} , a simple differential equation for M_{atm} (or, equivalently, P) results, whose solution, converted to a convenient and universal form, is:

$$\frac{M_{\text{atm}}(t)}{M_0} = \frac{P(t)}{P_0} = \left(1 - \frac{t}{t_*}\right)^{1/b} \quad (3)$$

where M_0 is the present ($t=0$) atmospheric mass, P_0 is the present atmospheric pressure and t_* is the length of time required for impacts to reduce the atmospheric pressure to zero. In terms of previously defined quantities,

$$t_* = \frac{1}{2\pi ab(RH)^{1-b}} \left(\frac{2\pi P_0}{g}\right)^b \quad (4)$$

Note that the above equation for the time dependence of atmospheric mass or pressure can be used for times before the present ($t < 0$), so that the atmospheric pressure at any previous era can also be computed. It is interesting to note that the atmospheric pressure declines rigorously to zero at time t_* —it does not simply fall exponentially towards zero. This interesting fact (K. Zahnle, personal communication) is a unique characteristic of the impact erosion mechanism. As the atmospheric pressure declines, smaller projectiles are capable of removing the atmosphere above the tangent plane. But by the distribution law, there are more smaller projectiles than larger ones, so a greater fraction of the atmosphere is removed in each unit time interval. The net result is the complete stripping of the atmosphere after time t_* , barring volcanic or other sources of replenishment.

The post-late-heavy-bombardment flux, along with other parameters, gives a value of $t_* \approx 60$ Gyr for Mars at the present time (note that t_* for the Earth and Venus is longer by several orders of magnitude, so that the impact erosion of their atmospheres is entirely negligible). Although this number is quite uncertain, it should probably be regarded as an upper limit, as the enhancement of vaporization by oblique impact and a possible large admixture of cometary impacts in the martian cratering flux both tend to shorten t_* . The adopted value for t_* predicts an atmospheric pressure at the end of late heavy bombardment (3.2 Gyr) only 1.1 times the present low pressure. This would not explain the geomorphic evidence¹² for running water on Mars's surface early in its geological history. It is widely known⁷, however, that the impact flux on the Moon in the first 10^9 years

of its history was many orders of magnitude larger than at present. It is presumed¹¹ that Mars, too, shared in this era of 'heavy bombardment', so that the enhanced impact fluxes of this era may have produced an early period of rapid atmospheric-pressure change. The heavy bombardment flux can be adequately described by

$$N_{\text{cum}}(m, t) = a(1 + B e^{-\lambda(t+4.6)})m^{-b} \quad (5)$$

where a and b are the same as before, $B = 2,300$ and $\lambda = 4.53 \text{ Gyr}^{-1}$. Figure 2 shows that the crater density computed by this expression provides an excellent fit to the lunar data as far back as 4.4 Gyr.

The differential equation for the rate of atmospheric mass loss implied by equation (5) can be readily integrated, giving a simple analytic expression similar to equation (3) for the evolution of atmospheric pressure as a function of time t from the present:

$$\frac{M_{\text{atm}}(t)}{M_0} = \frac{P(t)}{P_0} = \left(1 - \frac{t}{t_*} - \frac{B e^{-4.6\lambda}}{\lambda t_*} [1 - e^{-\lambda t}]\right)^{1/b} \quad (6)$$

The atmospheric pressure of Mars predicted by this expression is shown in Fig. 3. It is clear that early in Mars's history its atmospheric pressure must have been at least 100 times its present value, that is, ~ 1 bar. Note that this pressure is near that considered necessary for Mars to have supported free water on its surface¹³.

The simple analytical model of atmospheric erosion described here clarifies several aspects of martian climatic history. First, it is important to emphasize that the present atmosphere of Mars is stable against atmospheric erosion on the timescale of several tens of Gyr. Second, the impact flux during the era of late heavy bombardment was so high that Mars must have had a primordial atmospheric pressure near 1 bar: otherwise, its present atmospheric pressure would be even lower than it is now. This observation agrees well with the geomorphic evidence for fluvial processes during the era of heavy bombardment and with the more indirect evidence¹⁴ for an early era of enhanced erosion that erased small craters and obliterated the rims and other details of larger ones. Mars was able to accumulate its primordial atmosphere in the first place because impact velocities during accretion were too low for the vapour plumes to reach escape velocity. Note that impact erosion does not fractionate atmospheric constituents: a sufficiently large, fast impact removes the atmosphere completely, preserving the ratios of gaseous species while reducing their absolute abundance. However, a condensable substance such as water might be preserved on the surface while other atmospheric constituents are ejected. This property of impact erosion may go far in explaining some of the apparent differences in the atmospheric rare-gas inventories of Mars and the Earth. Our model makes it clear that of all the terrestrial planets, Mars is peculiarly susceptible to impact erosion because of its small radius and low gravitational acceleration. Application of the same model to the Earth indicates a primordial atmospheric pressure about 6 times its present value, whereas Venus would have had an initial atmosphere of only ~ 1.5 times its present pressure. $\square$

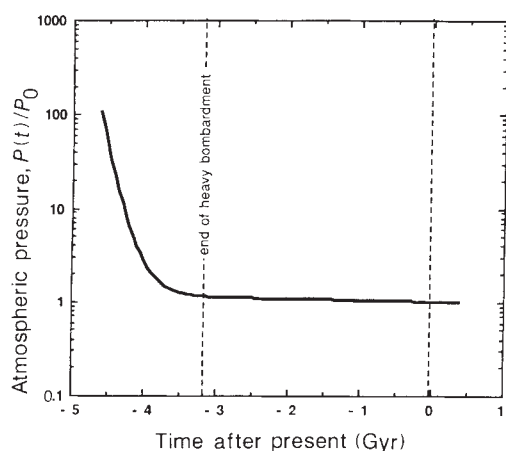


FIG. 3 Evolution of the atmospheric pressure $P(t)$ on Mars relative to its present value P_0 . This figure is constructed using equation (6) assuming $t_* = 63.1$ Gyr, $B = 2,300$ and $\lambda = 4.53 \text{ Gyr}^{-1}$. It shows the rapid decline in Mars's atmospheric pressure throughout the era of late heavy bombardment.

Received 19 October 1988; accepted 8 February 1989.

1. Walker, J. G. C. *Icarus* **68**, 87–98 (1987).
2. Ahrens, T. J. & O'Keefe, J. D. *Int. J. Impact Engng.* **5**, 13–32 (1987).
3. Melosh, H. J. & Vickery, A. M. *Eos* **69**, 388 (1988).
4. Cameron, A. G. W. *Icarus* **56**, 195–201 (1983).
5. Watkins, G. H. thesis, Mass. Inst. Tech. (1983).
6. Melosh, H. J. *Impact Cratering* (Oxford University Press, 1989).
7. BVSP, *Basaltic Volcanism on the Terrestrial Planets* (Pergamon, New York, 1981).
8. Hartmann, W. K. *Icarus* **31**, 260–276 (1977).
9. Schmidt, R. M. & Housen, K. R. *Int. J. Impact Engng.* **5**, 543–560 (1987).
10. Strom, R. G. in *The Geology of the Terrestrial Planets*, (ed. Carr, M.) (NASA SP-469, US Government Printing Office, Washington, DC, 1984).
11. Neukum, G. & Wise, D. U. *Science* **194**, 1381–1387 (1976).
12. Baker, V. R. & Partridge, J. B. *J. geophys. Res.* **91**, 3561–3572 (1986).
13. Cess, R. D., Ramanathan, V. & Owen, T. *Icarus* **41**, 159–165 (1980).
14. Chapman, C. R. & Jones, K. L. A. *Rev. Earth planet. Sci.* **5**, 515–540 (1977).

Relation between increasing methane and the presence of ice clouds at the mesopause

Gary E. Thomas*, John J. Olivero†, Eric J. Jensen*, Wilfred Schroeder‡ & Owen B. Toon§

* Laboratory for Atmospheric and Space Physics and Department of Astrophysical, Planetary and Atmospheric Sciences, University of Colorado, Boulder, Colorado 80309, USA

† Department of Meteorology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

‡ Geophysical Station, 282 Bremen-Boennebeck, Hechelstrasse 8, FRG

§ Space Science Division, NASA-Ames Research Center, Moffett Field, California 94035, USA

TRENDS of increasing atmospheric methane, carbon dioxide and other species have now been identified¹. It is well-known that water vapour is an important product of methane oxidation in the stratosphere^{2,3} and here we investigate the possibility that a substantial change has occurred in middle-atmospheric water vapour as a result of the increase in methane over the past century and a half. We show from modelling of mesospheric ice-particle formation that noctilucent cloud brightness should be a sensitive indicator of the water content at the high-latitude summertime mesopause (at a height of 85 km). Blake and Rowland⁴ have recently suggested that the occurrence of polar stratospheric clouds may be increasing because of increasing methane. We look at the record of noctilucent cloud occurrence for which the historical record is more complete. We find that noctilucent clouds are absent from the historical record before 1885, which is consistent with our hypothesis.

The hypothesis that upper atmospheric water vapour is increasing because of increases in methane is testable by the consequent effect on noctilucent-cloud (NLC) brightness, which has been recorded for over a century. Our model⁵ of NLC ice-particle formation and growth shows quantitatively that cloud brightness may indeed be a sensitive indicator of water-vapour concentration. In view of this, it is remarkable that, despite the fact that observations of NLCs have been made almost every year since their discovery in 1885, none were observed before this date, notwithstanding the attention of a number of skilled observers, located at the appropriate latitudes, who were sufficiently familiar with twilight phenomena. Using our NLC model, we show that clouds were probably not detect-

able in previous centuries because of their weakness compared with the twilight background. Alternatively, however, the discovery of NLCs may have been prompted by the Krakatoa volcanic eruption two years before, which would have caused volcanic injection of water into the stratosphere, followed by slow upward transport to the summertime mesopause. Nevertheless, we expect that the principal cause of most modern NLCs is the current high level of methane-derived water vapour.

Evidence that pre-industrial methane levels were about one half that of the present comes from measurements of reduced methane concentrations within air bubbles trapped in polar ice⁶. Biological sources of methane include anaerobic bacterial fermentation in rice fields, natural wetlands, landfills, domesticated animals (ruminants), and termites. Non-biological sources are natural-gas leaks and venting, mining activities, and industrial activities of several types. The major sink of atmospheric methane is the reaction with the hydroxyl radical (OH). Although the methane budget is not well understood⁷, the globally rising trend is probably a consequence both of increasing source strength (because of increased land use and increasing human and animal populations) and of a global reduction in atmospheric OH levels⁸.

Methane is nearly fully mixed in the troposphere because of its long lifetime (~ 8 years)⁷. Tropospheric gases are believed to enter the stratosphere in the tropics⁹, although important details of the processes involved are not yet understood¹. Distribution of methane throughout the stratosphere and mesosphere takes about 2–3 years (ref. 10).

At present, methane oxidation accounts for about one half of the total water above the tropopause¹¹. We define total hydrogen as $[H]_t = [H_2O] + 2[CH_4] + [H_2]$, where the bracket [] denotes mole fraction or mixing ratio. As there are no sources or sinks of $[H]_t$ in the upper stratosphere and mesosphere (height of 30–90 km), it is a conserved quantity throughout this region. Thus it is possible to infer changes in water vapour at the high-latitude summer mesopause without the use of a detailed model of atmospheric transport.

Stratospheric methane is slowly removed by complex reactions with OH and with excited atomic oxygen, $O(^1D)$, resulting in the production of water, molecular hydrogen and negligible amounts of odd-hydrogen radicals¹². The expectation that roughly two H_2O molecules are produced for every CH_4 molecule destroyed has been confirmed by satellite observations of water vapour and methane¹¹. Methane oxidation occurs mainly above 30 km, and repartitions $[H]_t$ primarily into H_2O

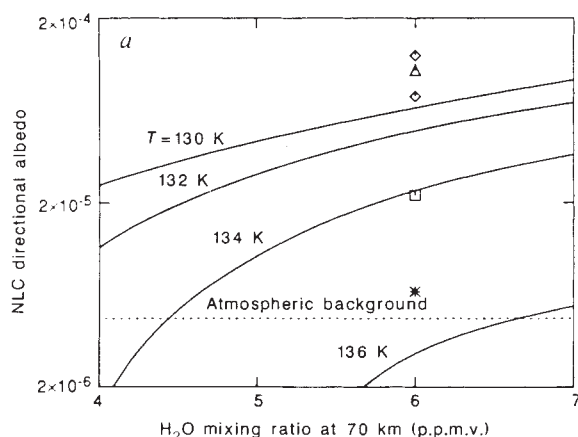
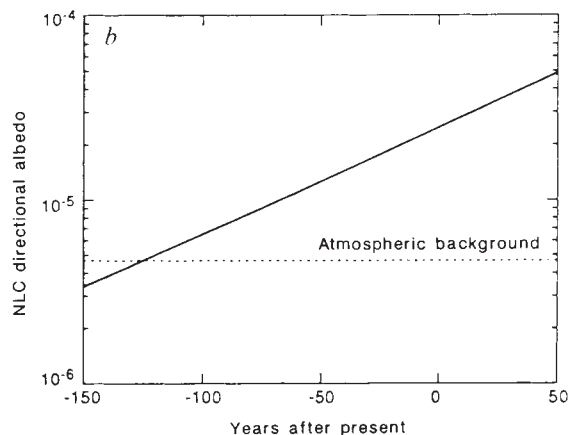


FIG. 1 *a*, Noctilucent cloud brightness versus water vapour mixing at the lower boundary of the microphysical model. Brightness is measured in terms of the directional albedo³¹, that is, the apparent emission rate divided by the solar flux at a wavelength of 555 nm. The elevation angle of observation and the solar depression angle are both assumed to be 11°. The four curves apply to different values of the mesopause temperature minimum: $T_M = 130, 132, 134$ and 136 K. The Rayleigh-scattered background albedo corresponds



to the viewing conditions assumed in the NLC albedo calculations. Also shown are measurements of the current NLC brightness (Δ ref. 32; $\diamond$ ref. 33; $\square$ ref. 34; * ref. 35). *b*, Noctilucent cloud brightness versus time, in years relative to the present. The values of water vapour were taken from Table 1, and the corresponding values of cloud brightness were taken from the 134-K curve of *a*.

and H_2 . In the high-latitude summer, $[H]_t$ consists almost entirely of H_2O , at concentrations of about 6 p.p.m. by volume up to about 70 km. A pronounced vertical gradient of $[H_2O]$ occurs in the upper mesosphere through the progressive photolytic destruction of H_2O (ref. 13). In this region, H_2 is formed from photolytic reactions among the products of water-vapour dissociation, which consist of odd-hydrogen free radicals. At the high-latitude summer mesopause, the partitioning of H_2O and H_2 is predicted¹² to be about equal, at 2 p.p.m.v.

With a current atmospheric mixing ratio of ~ 1.65 p.p.m.v., CH_4 contributes about 55% of the total hydrogen¹¹. The remaining 2.7 p.p.m.v. (or 45%) is derived from tropospheric/oceanic sources of H_2O . Assuming that the latter sources are constant in time, we may calculate the time variation of the water-vapour mixing ratio at 70 km that results from increasing methane levels. Table 1 shows the results calculated using the methane levels reported from ice bubble analyses⁶. As recently as 100 years ago, the H_2O level may have been only 4.5 p.p.m.v., as opposed to the current value of 6 p.p.m.v.

The current levels of H_2O —6 p.p.m.v. at 70 km and ~ 2 p.p.m.v. at the high-latitude summer mesopause—are sufficient to explain NLC brightness on the basis of particle growth modelling¹⁴. It is necessary that the mesopause temperature, T_M , be less than 135 K so that supersaturation conditions are met at these very low humidities¹⁵. Water-ice particles are nucleated near the mesopause by meteoric dust and/or by heavy-water cluster ions¹⁴. Several factors control the NLC brightness in our one-dimensional model, namely temperature and pressure at the mesopause, water-vapour mixing ratio, vertical diffusion rate, and vertical wind speed⁵. Unfortunately, none of these properties is well-known, and in addition they are probably variable from day to day. They must be treated as parameters in the model. Most relevant here is the dependence of cloud brightness on amount of water vapour and on mesopause temperature.

Shown in Fig. 1a are the model calculations of maximum NLC albedo plotted for a range of water-vapour mixing ratios at the lower boundary height of 70 km. We calculate H_2O concentrations above the lower boundary height by a combination of advective and diffusive transport, photolysis, and sublimation onto and from ice particles. The various curves correspond to different values of T_M , selected to fall within the observed range. Note that $[H_2O]$ at the observed height of NLCs (83 km) will be appreciably less than 6 p.p.m.v., because of solar photodissociation and depletion by growing ice particles. Also indicated in Fig. 1a is the range of NLC albedos reported in the literature.

For fixed T_M the model cloud brightness is very sensitive to $[H_2O]$, changing by more than an order of magnitude over a small range in humidity. This sensitivity is caused by the increase in particle growth rate, and resulting maximum particle size, with increasing H_2O concentration. The scattered radiance depends strongly on the effective particle radius r (being proportional to r^α , with $\alpha \approx 5-6$). These factors combine to yield a cloud radiance that scales as $[H_2O]^\beta$, with $\beta \approx 2-3$ (ref. 5). However, this scaling law was derived by assuming a fixed number of cloud particles, n . In our model, we use a nucleation scheme¹⁴ in which ice deposition occurs only on dust particles whose radius exceeds a critical value. For a fixed temperature, this critical radius decreases with increasing H_2O concentration. Because the concentration of dust particles increases sharply with decreasing radius, a small increase in H_2O concentration results in a large increase in the number of particles nucleated. The combined effects of increasing both the numbers and the sizes of cloud particles explains the strong sensitivity of NLC brightness to $[H_2O]$. This new model yields values of the exponent, β , that vary between 4 and 8, depending on various model parameters.

Based on the trend of $[H_2O]$ in Table 1, the evolution of NLC brightness with time is shown in Fig. 1b, along with the calculated Rayleigh-scattered background albedo. We show the results for

$T_M = 134$ K only, but the same general behaviour would be seen at other temperatures.

We now ask whether NLCs were likely to have been present but unnoticed in earlier years. The first observation of NLCs was made by Backhouse¹⁶ on 8 June 1885 in Bad Kissingen, Germany. Some days later this phenomenon came to the attention of numerous astronomers and meteorologists throughout Europe, Great Britain¹⁷ and in Russia¹⁸. Certain individuals confessed explicitly to have never seen NLCs before this date¹⁹⁻²⁷. Some reports of twilight enhancements before 1885 may be attributed to the phenomenon of 'bright nights', that is, enhanced airglow emission, which has been seen at all latitudes and seasons.

Evidence that NLCs were not observed before 1885 is not conclusive. If, however, a pre-1885 sighting of NLCs were to be confirmed, our hypothesis would not necessarily be damaged; we argue only that such sightings were much less likely than they are today. In fact, we believe that the very first sighting was probably not due to the slow emergence of NLCs above the sky background, but that the upper atmosphere probably received an added 'boost' of condensation nuclei and/or water vapour from the Krakatoa volcanic eruption in 1883. The two-year time delay is consistent with the time needed to transport material from the stratosphere to the upper mesosphere²⁸, and has been verified by recent model calculations¹⁰. From Fig. 1a, an abrupt enhancement in NLC brightness would require an injection of 1-2 p.p.m.v. of H_2O . Assuming that the water was injected at the observed height of the Krakatoa dust plume, a 1-2 p.p.m.v. increase corresponds to a total additional water mass of 100-200 megatons. (The mass of sulphate injected into the stratosphere by Krakatoa has been estimated to be 100 megatons^{36,37}.)

We believe that most of the NLC sightings over the past century can be ascribed to the increased water levels arising from methane oxidation. The periodic rise and fall of NLC activity on shorter timescales (decades) is probably due to shorter-term fluctuations in the controlling factors, the most important being material supplied by volcanic injections²⁹.

A direct and important inter-relationship between methane, water vapour and the existence of ice clouds in the middle atmosphere is plausible, but what are the long-term implications? In Table 1 and Fig. 1b we have extrapolated forward in time the curves representing water vapour and NLC brightness. We expect brighter and more widespread displays with longer seasons of occurrence. In fact, Gadsden³⁰ suggests, on the basis of observations made over the last three decades, that both NLC occurrence frequency and the duration of the NLC 'season' have increased. However, the trend that we would predict over this time interval is much less dramatic than Gadsden's. Furthermore, the time series used in ref. 30, derived from observations in northern Europe, does not agree well with corresponding

TABLE 1 Estimated time history of tropospheric methane and high-latitude mesospheric water vapour

Years (before present)	Methane surface concentration (p.p.m.v.)	Water vapour at 70 km (p.p.m.v.)
150	0.8	4.3
100	0.9	4.3
100	0.9	4.5
50	1.2	5.2
0	1.65	6.0
-50	2.7	8.1

The trends of methane up to the present time is taken from Fig. 1 of ref. 6. Mesospheric water vapour is assumed to originate from the atmospheric/oceanic source of 2.7 p.p.m.v. plus a source from methane oxidation (one CH_4 molecule produces two H_2O molecules). At 70 km, total hydrogen $[H]_t$ is assumed to be primarily in the form of $[H_2O]$. The future trend for methane is taken to be 1.3% per year.

data from the USSR, so that the reported trend may be of regional but not global significance.

It is unlikely that the current atmospheric structure and dynamics will remain unchanged in the face of the important compositional changes taking place throughout the atmosphere. In particular, reductions in ozone and increases in carbon dioxide will alter both the thermal and dynamic structure of the entire middle atmosphere. As noctilucent clouds are probably under strong dynamic control, even small perturbations could be important.

We conclude the following: (1) the first manifestation of the noctilucent cloud phenomenon occurred in the summer of 1885

in northern Europe; (2) before 1885 there was insufficient water vapour routinely available at the mesopause to form visible clouds; (3) the NLC sightings during the period 1885–1895 were probably made possible in part by the large stratospheric injections of water vapour that resulted from the Krakatoa eruption in 1883; (4) the increase in water from methane oxidation will continue in the immediate future, increasing the occurrence frequency and brightness of NLC; and (5) in the long term, the continued rise of trace substances in the atmosphere will act to change the structure and transport within the middle atmosphere in ways that are extremely difficult to predict, making the future of NLC behaviour uncertain. □

Received 17 October 1988; accepted 24 February 1989.

1. World Meteorological Organization, Global Ozone Research & Monitoring Project—Report No. 16 *Atmospheric Ozone 1985: Assessment of our Understanding of the Processes Controlling its Present Distribution and Change* (1985).
2. Singer, S. F. *Nature* **233**, 543–545 (1971).
3. Jones, R. L. & Pyle, J. A. *J. geophys. Res.* **89**, 5263–5279 (1984).
4. Blake, D. R. & Rowland, F. S. *Science* **239**, 1129–1131 (1988).
5. Jensen, E. & Thomas, G. E. *J. geophys. Res.* **93**, 2461–2473 (1988).
6. Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **89**, 11599–11605 (1984).
7. Khalil, M. A. & Rasmussen, R. A. *J. geophys. Res.* **88**, 5131–5144 (1983).
8. Thompson, A. M. & Cicerone, R. J. *J. geophys. Res.* **91**, 10853–10864 (1986).
9. Brewer, A. W. *Q. J. R. met. Soc.* **75**, 351–363 (1949).
10. Callis, L. B., Boughner, R. E. & Lambeth, J. D. *J. geophys. Res.* **92**, 5585–5607 (1987).
11. Jones, R. et al. *Q. J. R. met. Soc.* **112**, 1127–1143 (1986).
12. LeTexier, H., Solomon, S. & Garcia, R. R. *Q. J. R. met. Soc.* **114**, 281–295 (1988).
13. Tsou, J.-J., Olivero, J. J. & Crosky, C. L. *J. geophys. Res.* **93**, 5255–5266 (1988).
14. Turco, R. P., Toon, O. B., Whitten, R. C., Keesee, R. G. & Hollenback, D. *Planet. Space Sci.* **30**, 1147–1181 (1982).
15. Garcia, R. R. *J. geophys. Res.* (in the press).
16. Backhouse, T. W. *Met. Mag.* **20**, 133 (1885).
17. Leslie, R. C. *Nature* **33**, 264 (1886).
18. Tseraskii, V. K. *Astronomicheskii fotometr i ego prilozheniya Matemat. sbornik* **13**, 76–81 (1887).
19. Jesse, O. *Met. Z.* **5**, 90–94 (1888).
20. Hartwig, E. *Ber. Naturforsch. Ges. Bamberg* **VXI**, 1–4 (1893).
21. Kiessling, J. *Sitz-Ber. Kgl. Akad. d. Wiss. Berlin* **XXX-LII**, 529–533 (1886).

22. Fritz, H. *Die wichtigsten periodischen Erscheinungen der Meteorologie und Kosmologie* (Brockhaus, Leipzig, 1889).
23. Riggelbach, A. *Beobachtungen über die Dämmerung, insbesondere über das Purpurlicht und seine Beziehungen zum Bishop'schen Sonnenring* (Georgs, Basel, 1886).
24. Pernter, J. *Met. Z.* **6**, 447–466 (1889).
25. Voith, C. thesis, Univ. Erlangen (1909).
26. Tseraskii, V. K. *Trudy Moskovskoi observatorii Ser. II* Vol. 2 (1890).
27. McConnell, D. *Met. Mag.* **117**, 87–93 (1988).
28. Schröder, W. *Geowiss. in unserer Zeit* **1**, 155–159 (1983).
29. Fogle, B. & Haurwitz, B. *Bonn Univ. Clim. Res.* (eds Fraedrich, K., Hantel, M., Korss, H. C. & Ruprecht, E.) 263–276 (Ferd. Dümmler Verlag, Bonn, 1973).
30. Gadsden, M. in *Collections of Works of the International Workshop of Noctilucent Clouds* (ed. Avaste, O.) (Tallin, Estonia SSR, USSR, 1984).
31. Thomas, G. E. & McKay, C. P. *Planet. Space Sci.* **33**, 1209–1224 (1985).
32. Fogle, B. & Rees, M. H. *J. geophys. Res.* **77**, 720–725 (1972).
33. Tozer, W. F. & Beeson, D. E. *J. geophys. Res.* **79**, 5607–5612 (1974).
34. Veselov, D. P., Popov, O. I., Semyanova, V. I., Seleznev, G. I. & Fedorova, Ye. O. *Atmos. Ocean Phys.* **12**, 1097–1099 (1976).
35. Witt, G., Dye, J. E. & Wilhelm, N. *J. atmos. terr. Phys.* **38**, 223 (1976).
36. Legrand, M. & Delmas, R. *J. Nature* **327**, 671–676 (1987).
37. Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288**, 230–235 (1980).

ACKNOWLEDGEMENTS. This research was supported by the Aeronomy Program of the Atmospheric Sciences Division of NSF and by the Innovative Science and Technology Program of the Office of Naval Research. O.B.T. was supported by NASA's Upper Atmosphere Theory Program.

Limitations on the rapid reduction of nitrogen oxides in exhaust gas streams

Brian G. Wicke, Karen A. Grady & John W. Ratcliffe

Physical Chemistry Department, General Motors Research Laboratories, Warren, Michigan 48090, USA

ALL commercially important combustion systems produce exhaust gases containing substantial amounts of nitrogen oxides and molecular oxygen. Because of the part played by nitrogen oxides in smog formation and acid-rain production, methods of reducing NO_x emissions have been subject to intense research. In 1986, Perry and Siebers¹ proposed a new chemical method for removing NO_x from diesel exhaust gas by adding cyanuric acid (HNCO)₃, and suggested that this new process, called RAPRENOx (rapid reduction of NO_x) could control the emission of NO_x from most combustion devices. Here we show that surface chemistry is responsible for the effective reduction of NO_x , confirming recent results². In stainless steel, in the absence of oxygen, NO is effectively reduced by cyanuric acid at 700 °C. When oxygen is present, however, NO is produced by the reaction with cyanuric acid at 700 °C. Under conditions where surface effects are important (such as in the first demonstration experiments reported on RAPRENOx) the reduction of NO by cyanuric acid is adversely affected by oxygen.

To learn more about the chemistry involved in RAPRENOx, we carried out a series of laboratory flow-tube experiments under conditions similar to those reported initially¹. We used two different flow tubes, one quartz and the other stainless steel; except for this difference in material, the two flow tubes were identical (22 mm inner diameter, 80 cm long). Pure gases (Ar, NO, CO, CO₂, O₂, H₂, N₂ and N₂O) were admitted to the flow tube through calibrated electronic flow controllers. The flow tube has two independently controlled heated sections: a pre-

heater section (20 cm long) and a reaction section (25 cm long) immediately downstream. Cyanuric acid was sublimed from a quartz crucible lowered into the centre of the preheater region; adjusting the preheater temperature provided independent control of the cyanuric acid sublimation rate.

A mass spectrometer sampled the gas flow downstream of the reaction region. The mass spectrometer sensitivity for the gases of interest was measured before and after each experiment using known flows of the pure gases. Water was also observed in these experiments; because of problems related to H₂O condensation on the flow tube walls, no attempt was made to measure H₂O quantitatively. Ar was the predominant gas (>95%). Typical conditions were: pressure 28 kPa; reactor temperature 700 °C; residence time at 700 °C = 1 s. These conditions were chosen to approximate those of the earlier study¹.

In the quartz flow tube, most of the cyanuric acid which sublimed in the preheater region deposited out onto the cool flow-tube walls downstream of the 700 °C reaction. A small fraction of the cyanuric acid did decompose; the observed decomposition products included HNCO, CO, CO₂, N₂, H₂O, NH₃ and HCN. Addition of NO upstream of the cyanuric acid produced no change in the thermal decomposition of cyanuric acid, and no reduction of NO was observed.

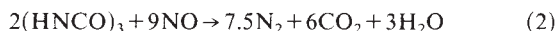
In the otherwise equivalent stainless-steel flow tube, cyanuric acid decomposed rapidly at 700 °C; no solid deposits were observed on the flow-tube walls. The decomposition products were CO, H₂ and N₂, and a small amount of CO₂. We analysed the mass spectrometer data and determined the overall stoichiometry of this fast thermal decomposition in stainless steel as:



The small amount of CO₂ observed results at least in part from the oxidation of CO on the walls of the heated flow tube. The independently measured sublimation of cyanuric acid agreed well with the amounts of H, N and C measured in H₂, N₂ and CO+CO₂.

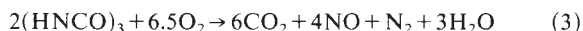
In stainless steel at 700 °C, we observed the rapid reduction

of NO by cyanuric acid. The chemistry is represented by the following stoichiometry:



All species were measured independently except H_2O . No N_2O was observed. The 1-s residence time at 700°C was sufficient for complete reaction. These observations of the importance of surface chemistry confirm recent findings². Experiments to identify the role of the surface interactions are in progress.

We also find that cyanuric acid reacts rapidly with O_2 at 700°C in stainless steel. Our quantitative measurements are consistent with the following overall stoichiometry:



This does not represent the molecular kinetics, of course; it is simply a balanced relationship between major reactants fed into the reactor and major products observed downstream. NO is the major nitrogen-containing product.

The competition for limiting cyanuric acid between NO and O_2 is illustrated by the sequence of mass spectra shown in Fig. 1. The top mass spectrum is for gas flow of $5.0 \text{ standard cm}^3 \text{ min}^{-1}$ of NO and $2.0 \text{ standard cm}^3 \text{ min}^{-1}$ O_2 in argon at 700°C in stainless steel. The centre mass spectrum shows the downstream species observed upon addition of $5.0 \text{ standard cm}^3 \text{ min}^{-1}$ NO to limiting cyanuric acid sublimation; note the excess NO at 30 AMU. The bottom mass spectrum shows the addition of $2.0 \text{ standard cm}^3 \text{ min}^{-1}$ O_2 to this NO/limiting cyanuric acid flow. Oxygen is consumed (32 AMU) and more NO is observed than was input (top). In limiting cyanuric acid, O_2 reacts more rapidly than NO under these conditions.

This seems to contradict the original diesel experiment which purported to demonstrate that RAPRENOx was effective in a stainless-steel system in the presence of excess oxygen¹. We believe, however, that the diesel data support the O_2 sensitivity

we observe. In the diesel experiment, NO increased by a factor of at least 25 as the cyanuric acid bed temperature was raised to $\sim 340^\circ\text{C}$; further increase in the acid-bed temperature produced a rapid decrease of NO. As the acid-bed temperature was raised, cyanuric acid sublimed more rapidly and more reactant was entrained into the gas stream. Starting with limiting cyanuric acid in the oxygen-containing exhaust stream, NO was produced according to reaction (3); increasing the cyanuric-acid sublimation rate produced more NO by reaction (3) until the oxygen (which could not be detected by the Fourier transform infrared technique used¹) was consumed. Subsequent further increase in the cyanuric acid sublimation rate led to a decrease in the total NO by reaction (2).

For the conditions we have examined and the conditions originally reported in support of RAPRENOx¹, surface effects are responsible for rapid reduction of NO by cyanuric acid. Under these conditions, however, NO is produced when O_2 is present. D. L. Siebers and J. A. Caton (personal communication), as well as Heap *et al.*², have reported NO reduction using cyanuric acid under conditions where the surface effects examined here are not important. This gas-phase NO reduction chemistry is sensitive to temperature and the presence of other gases in the exhaust (ref. 2; D. L. Siebers and J. A. Caton, personal communication). The use of the RAPRENOx technique to control NO_x emissions seems therefore to be considerably more limited than was suggested originally¹. □

Received 19 September 1988; accepted 21 February 1989.

1. Perry, R. A. & Siebers, D. L. *Nature* **324**, 657–658 (1986).

2. Heap, M. P., Chen, S. L., Kramlich, J. C., McCarthy, J. M. & Pershing, D. W. *Nature* **335**, 620–622 (1988).

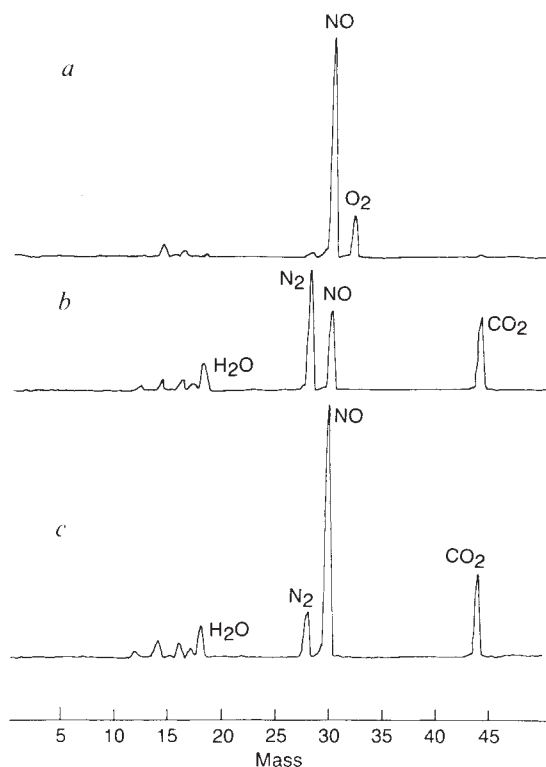


FIG. 1 Mass spectra showing the competition for limiting cyanuric acid between NO and O_2 at 700°C in stainless steel. a, $\text{NO} = 5.0 \text{ standard cm}^3 \text{ min}^{-1}$, $\text{O}_2 = 2.0 \text{ standard cm}^3 \text{ min}^{-1}$, no cyanuric acid sublimation. b, $\text{NO} = 5.0 \text{ standard cm}^3 \text{ min}^{-1}$, no O_2 , and stable limiting cyanuric acid sublimation. c, Same as b, to which $2.0 \text{ standard cm}^3 \text{ min}^{-1}$ O_2 has been added.

Glacial–Holocene salinity changes in the Mediterranean Sea: hydrographic and depositional effects

Robert C. Thunell & Douglas F. Williams

Department of Geological Sciences, University of South Carolina, Columbia, South Carolina 29208, USA

OXYGEN isotope changes as recorded by Pleistocene foraminifera from open-ocean sediments are primarily a function of changes in the isotopic composition of the global seawater reservoir caused by ice-volume changes, with a secondary temperature component¹. Oxygen isotope records from marginal basins such as the Gulf of Mexico², the Mediterranean Sea^{3–5} and the Red Sea⁶ also exhibit the global signal but are far more complicated owing to the strong overprint of local climate conditions. In particular, glacial–interglacial changes in continental aridity and river runoff produce significant salinity changes in these semi-enclosed basins. Using available oxygen isotope records we estimate that glacial (18,000 yr BP) salinities in the eastern and western Mediterranean Sea were 2.7‰ and 1.2‰ higher, respectively, than at present. These elevated glacial salinities played an important part in preconditioning the eastern Mediterranean for the eventual accumulation of organic carbon-rich sediments (sapropels) at the end of deglaciation.

Our database consists of thirty published planktonic foraminiferal oxygen isotope records from the eastern and western basins of the Mediterranean (Table 1; refs 7–17). For each record, the magnitude of the oxygen isotope change has been estimated for three different intervals: (1) from 18,000 yr BP to present, (2) from 18,000 yr BP to 8,000 yr BP, and (3) from 8,000 yr BP to present (Table 1). The 8,000 yr BP level represents

the midpoint (in time) of deposition of the most recent sapropel⁸. For each of these time intervals we estimate how much of the observed isotopic change can be attributed to salinity change (ΔS) using the following equations:

$$\Delta S_1 = (\Delta 18k-P) - V - \Delta T_1 \quad (1)$$

$$\Delta S_2 = (\Delta 18k-8k) - V - \Delta T_2 \quad (2)$$

$$\Delta S_3 = (\Delta 8k-P) - V - \Delta T_3 \quad (3)$$

where ΔS_1 , ΔS_2 and ΔS_3 are $\delta^{18}O$ changes (‰) owing to salinity changes for the intervals 18,000 yr BP–present, 18,000 yr BP–8,000 yr BP and 8,000 yr BP–present, respectively. $\Delta 18k-P$, $\Delta 18k-8k$ and $\Delta 8k-P$ are the calculated $\delta^{18}O$ changes for the three time intervals and V is the glacial-interglacial ice volume contribution to the $\delta^{18}O$ changes. ΔT_1 , ΔT_2 and ΔT_3 are the $\delta^{18}O$ changes attributable to temperature for the three time intervals.

To solve these equations we have assumed that deglaciation was virtually complete by 7,000–8,000 yr BP (refs 19, 20). Therefore, the ice-volume (V) contribution to the total $\delta^{18}O$ change in equations (1) and (2) is assumed to be the same, and the ice-volume component in equation (3) is assumed to be zero. We have also assumed that the warming of surface-water temperatures from glacial to present-day values in the eastern and western Mediterranean was complete by ~8,000 yr BP. This does not imply that there have been no temperature or climatic oscillations in the Mediterranean region during the last 8,000 yr. In fact temperatures were probably warmer during the thermal maximum at ~6,000 yr BP. What we have assumed is that there has been no significant net change in temperature between 8,000 yr BP and present. This assumption is supported by palaeotemperature estimates for the adjacent eastern North

Atlantic²¹. As a result, $\Delta T_1 = \Delta T_2$ and $\Delta T_3 = 0$. From these assumptions equation (3) becomes simplified to:

$$\Delta S_3 = \Delta 8k-P \quad (4)$$

which means that the entire difference in $\delta^{18}O$ between 8,000 yr BP and present is assumed to be due to salinity changes.

Using the data in Table 1, mean values have been determined for the isotopic changes associated with the three time intervals of interest for both the eastern and western Mediterranean (Table 2a; Fig. 1). The mean $\delta^{18}O$ changes between 18,000 yr BP and present are very similar, 3.1‰ and 2.9‰ for the eastern and western basins, respectively. In contrast, the isotopic differences between the eastern and western basins for the other two time intervals are relatively large. The $\delta^{18}O$ difference between 18,000 and 8,000 yr BP is 0.7‰ greater in the eastern basin (4.3‰) than in the western basin (3.6‰). Similarly, the $\delta^{18}O$ response between 8,000 yr BP and present is 0.4‰ larger in the eastern basin (–1.2‰) than in the western basin (–0.8‰) (Table 2a).

The salinity contribution to the observed oxygen isotope change between any two data points can be determined if reasonable estimates are available for the ice-volume effect and the magnitude of temperature change. The ice-volume effect for the most recent glacial-interglacial transition was between 1.1 and 1.3‰ (ref. 22). For our calculations, an ice volume (V) of 1.2‰ is used. Foraminiferal transfer-function estimates of glacial sea surface temperatures in the Mediterranean^{23,24} indicate that the western and eastern basins were on average ~6 °C and 4 °C colder than at present, respectively. Using the $\delta^{18}O$ –temperature relationship of 0.2‰ per °C (ref. 25) means that 1.2‰ and 0.8‰ of the total glacial-interglacial $\delta^{18}O$ changes (ΔT_1 and ΔT_2) in the western and eastern basins are a function of temperature.

TABLE 1 Planktonic foraminiferal oxygen isotope data for Mediterranean cores

Core number	Latitude	Longitude	18k (‰)	8k (‰)	Present (‰)	18k-P (‰)	18k-8k (‰)	8k-P (‰)	Ref.
Western Mediterranean									
82KS32	36°07' N	02°07' W	3.3		0.3	3.0			7
82KS31	36°09' N	03°16' W	3.2		0.5	2.7			7
82KS30	36°27' N	03°53' W	3.2		0.5	2.7			8
C68	35°41' N	04°05' W		–2.0	0.5			–2.5	9
82KS41	35°59' N	04°24' W	2.5		0.5	2.0			8
70KS05	38°06' N	02°59' E	3.2	–1.2	–1.0	4.2	4.4	–0.2	8
70KS06	38°31' N	04°00' E	3.2	–0.8	0.5	2.7	4.0	–1.3	10
C3	42°47' N	07°41' E	4.0	–1.6	–1.0	5.0	5.6	–0.6	11
KET8022	40°35' N	11°42' E	3.9	1.2	1.6	2.3	2.7	–0.4	12
70CS05	35°44' N	13°11' E	3.6	0.5	0.8	2.8	3.1	–0.3	5
KET8004	39°40' N	13°34' E	3.9	1.2	1.4	2.5	2.7	–0.2	12
KET8003	38°49' N	14°29' E	3.8	0.8	1.4	2.4	3.0	–0.6	12
Eastern Mediterranean									
KS09	35°09' N	20°09' E	3.3		–0.8	4.1			13
V10-67	35°42' N	20°43' E	3.0	–1.2	0.5	2.5	4.2	–1.7	8
TR171-24	34°03' N	22°32' E	4.1	1.2	2.5	1.6	2.9	–1.3	3
67M03	34°25' N	24°50' E	4.7	–0.6	1.4	3.3	5.3	–2.0	5
RC9-181	33°25' N	25°00' E	3.1	–1.2	0.1	3.0	4.3	–1.3	13
TR171-27	33°50' N	25°59' E		0.1	1.8			–1.7	3
CHN119-6	33°15' N	26°00' E	3.6	0.2	0.7	2.9	3.4	–0.5	4
75KS52	34°00' N	26°19' E	3.2	–0.8	0.0	3.2	4.0	–0.8	14
75KS50	34°41' N	27°00' E	3.6	–1.8	0.0	3.6	5.4	–1.8	5
ALB189	33°54' N	28°29' E	3.5						13
CHN119-16	33°14' N	30°19' E		–0.2	0.7			–1.0	4
CH119-18	34°20' N	30°53' E	4.6	0.1	0.7	3.9	4.5	–0.6	4
CH119-22	32°46' N	31°53' E		–0.6	0.9			–1.5	4
SH281-17	35°30' N	34°07' E	4.0	–1.1	–0.3	4.3	5.1	–0.8	15
GA32	31°57' N	34°21' E		–2.0	–1.0			–1.0	16
SH190-19	36°00' N	34°22' E	4.3	–0.5			4.8		15
82KS01	34°22' N	27°09' E	3.4		0.0	3.4			17
TR172-22	35°19' N	29°01' E	3.0	0.3	0.9	2.1	3.0	–0.6	3

The salinity component of the observed $\delta^{18}\text{O}$ changes has been calculated for each of the three time intervals using equations (1), (2) and (4) (Table 2b). To convert the salinity portion of the $\delta^{18}\text{O}$ change to salinity, we use the modern seawater oxygen-isotope/salinity relationship for the Mediterranean²⁶. According to this relationship, a 1‰ change in salinity will result in a 0.41‰ change in seawater $\delta^{18}\text{O}$. Using the present-day relationship between seawater oxygen isotopic composition and salinity to estimate past salinities is probably too simple. However, as it is not possible to define such a relationship for the past, using the modern relationship is the most reasonable solution to the problem.

The mean planktonic foraminiferal $\delta^{18}\text{O}$ changes between the last glacial maximum and the present in the western and eastern basins of the Mediterranean are 2.9‰ and 3.1‰, respectively (Table 2a; Fig. 1). These are considerably larger than the 1.8‰ change recorded in the Atlantic²⁷. If 1.2‰ of the mean change is due to ice-volume changes, $\delta^{18}\text{O}$ residuals of 1.7‰ for the western basin and 1.9‰ for the eastern basin represent the combined effect of temperature and salinity change. An additional 1.2‰ and 0.8‰ of the $\delta^{18}\text{O}$ residuals for the western and eastern basins are accounted for by glacial sea surface temperatures being 6 °C and 4 °C colder than at present^{23,24}. Of the total $\delta^{18}\text{O}$ changes that are observed between 18,000 yr BP and present, 0.5‰ and 1.1‰ are due to salinity changes in the western and eastern Mediterranean, respectively (Table 2b). These $\delta^{18}\text{O}$ changes are equivalent to salinity changes of 1.2‰ in the western basin and 2.7‰ in the eastern basin (Table 2b). Typical present-day surface salinities in the western and eastern Mediterranean are 38.2‰ and 38.8‰, respectively. From the oxygen isotope results, we estimate that salinities increased to 39.4‰ in the western basin and 41.5‰ in the eastern basin during the last glacial.

The mean $\delta^{18}\text{O}$ change between 18,000 yr BP and 8,000 yr BP is 3.6‰ for the western basin and 4.3‰ for the eastern basin (Table 2a, Fig. 1). Under the assumptions that deglaciation and surface-temperature warming were virtually complete by 8,000 yr BP, the salinity contribution to the observed $\delta^{18}\text{O}$ changes is 1.2‰ for the western basin and 2.3‰ for the eastern basin (Table 2b). These $\delta^{18}\text{O}$ changes represent salinity changes of 2.9‰ and 5.6‰ for the western and eastern Mediterranean, respectively (Table 2b). Using the salinities determined for 18,000 yr BP, we estimate that salinities at 8,000 yr BP were ~36.5‰ in the western basin and 35.9‰ in the eastern basin.

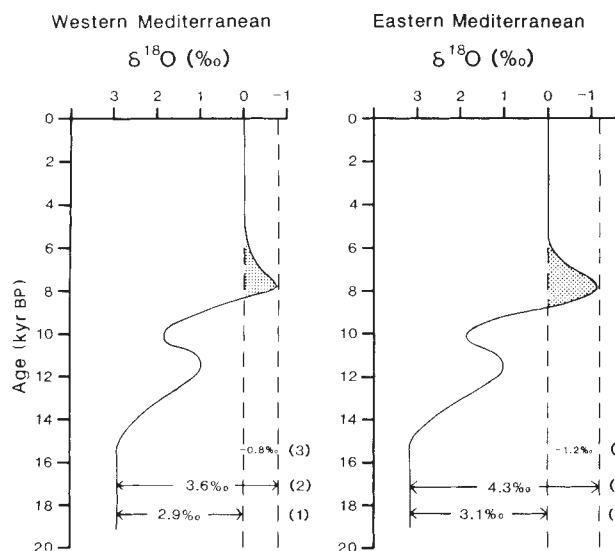


FIG. 1 Generalized planktonic foraminiferal oxygen isotope records for the western and eastern Mediterranean. The magnitudes of the isotopic differences between (1) 18,000 yr BP–present, (2) 18,000 yr BP–8,000 yr BP, and (3) 8,000 yr BP–present are indicated on each record.

TABLE 2 Oxygen isotope and salinity changes in the Mediterranean

a, Mean planktonic foraminifera		
Time interval	Western basin*	Eastern basin*
18,000 yr BP–present	2.9‰	3.1‰
18,000 yr BP–8,000 yr BP	3.6‰	4.3‰
8,000 yr BP–present	–0.8‰	–1.2‰

b, Oxygen isotope changes attributed to salinity and estimated salinity changes (ΔS)				
Time interval	Western basin		Eastern basin	
	$\delta^{18}\text{O}$ (‰)	ΔS (‰)†	$\delta^{18}\text{O}$ (‰)	ΔS (‰)†
18,000 yr BP–present	0.5	1.2	1.1	2.7
18,000 yr BP–8,000 yr BP	1.2	2.9	2.3	5.6
8,000 yr BP–present	–0.8	–1.9	–1.2	–2.9

* Based on data in Table 1.

† Derived from the seawater oxygen isotope/salinity relationship for the Mediterranean²⁶ whereby a 1.0‰ change in salinity produces a 0.41‰ change in $\delta^{18}\text{O}$.

As previously discussed, all of the observed $\delta^{18}\text{O}$ change between 8,000 yr BP and the present can be attributed to salinity. The –0.8‰ and –1.2‰ $\delta^{18}\text{O}$ changes for the western and eastern basins (Table 2b) are equivalent to salinity increases of 1.9‰ in the western basin and 2.9‰ in the eastern basin between 8,000 yr BP and the present.

The estimated salinity changes have a number of important implications for both the palaeohydrographic and depositional conditions in the Mediterranean since the last glacial period. The present arid conditions in the Mediterranean region create a negative water balance (in that evaporation exceeds precipitation and runoff) which results in an anti-estuarine exchange with the North Atlantic (Fig. 2a). Our isotopic results indicate that these conditions changed during the last glacial (Fig. 2c). The higher glacial salinities suggest that the water balance of the Mediterranean became more negative as the region became even more arid than at present. This is in agreement with pollen and lake-level records from the circum-Mediterranean. In particular, African lake levels were at a minimum during the last glacial²⁸ and pollen spectra indicate colder, drier conditions between 26,000 and 13,000 yr BP than today²⁹.

The isotopic evidence suggests that the west-to-east gradient in surface salinities was maintained during the last glacial, implying that surface and deep-water exchanges were similar to those of today (Fig. 2c). The Mediterranean was still exporting dense outflow water to the Atlantic during the last glacial³⁰, although the volume of outflow was considerably reduced due to eustatically controlled restriction at the Gibraltar sill²⁶. The salinity and density of glacial intermediate and deep waters in the Mediterranean would also have been elevated, because the sources of these water masses are simply Mediterranean surface waters. Because oxygen solubility decreases with increasing salinity, glacial deep waters in the Mediterranean probably had lower dissolved oxygen concentrations than at present.

Hydrographic conditions in the Mediterranean at 8,000 yr BP must have been considerably different than at 18,000 yr BP. The water balance became positive as precipitation and run-off exceeded evaporation (Fig. 2b). Salinities were considerably lower at 8,000 yr BP and the west–east salinity gradient was reversed to an east–west gradient. This is supported by East African climate records which indicate the onset of very humid conditions at ~12,500 yr BP, with wettest conditions occurring between 10,000–8,000 yr BP (refs 14, 28). This was also a time of intensified African monsoons and increased Nile discharge^{14,31}.

The reversal of the salinity gradient coupled with a positive water balance in the Mediterranean at 8,000 yr BP strongly suggests a reversal of the surface flow patterns, that is, surface waters flowed out of the Mediterranean (Fig. 2b). This reversal

of circulation patterns, previously proposed on the basis of sedimentological results³², may have begun as early as 12,000 yr BP with the onset of pluvial conditions in this region. Such a circulation change means that the Mediterranean was not a source of dense outflow to the Atlantic at this time.

As previously mentioned, 8,000 yr BP represents the midpoint of the deposition of the most recent sapropel (7,000–9,000 yr BP)¹⁸. Most workers have invoked versions of two general models for sapropel formation: a 'density-stratification' model and a 'productivity' model. The density-stratification model requires a low-salinity surface layer which inhibits bottom-water oxygen renewal and eventually results in anoxia³³. The productivity model simply requires a high organic-carbon flux to produce sapropels^{18,34}. Jenkins and Williams⁴ and Howell and Thunell³⁵ evaluated both of these models and concluded that both increased productivity and a cessation of bottom-water renewal were required in order for sapropel deposition to occur within a period of several thousand years.

We believe that the key to understanding the formation of the most recent sapropel in the eastern basin is the glacial $\delta^{18}\text{O}$ patterns in both basins. The $\delta^{18}\text{O}$ patterns strongly suggest that high salinities (surface and deep water) during the last glacial were an important factor in 'preconditioning' the Mediterranean for sapropel deposition. First, bottom-water oxygen concentrations would have already been reduced because of the decrease in oxygen solubility associated with the increase in salinity. Second, the very high salinities which existed before sapropel formation would have more easily allowed the establishment of a density stratification with the onset of pluvial conditions. In addition, the circulation reversal would have caused the eastern Mediterranean to become a nutrient trap, thus enhancing pro-

ductivity. The combined effects of these conditions explains the most recent sapropel formation in the eastern Mediterranean and the contemporaneous deposition of reduced but not anoxic hemipelagic sediments in the western Mediterranean³⁶. □

Received 27 October 1988; accepted 8 February 1989.

- Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39–55 (1973).
- Leverter, A., Williams, D. F. & Kennett, J. P. *Earth planet. Sci. Lett.* **59**, 11–17 (1982).
- Thunell, R. C. & Williams, D. F. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **44**, 23–39 (1983).
- Jenkins, J. A. & Williams, D. F. *Mar. Micropaleont.* **8**, 521–534 (1984).
- Vergnaud Grazzini, C., Devaux, M. & Znaidi, J. *Mar. Micropaleont.* **10**, 35–69 (1986).
- Deuser, W. G., Ross, E. H. & Waterman, L. S. *Science* **191**, 1168–1170 (1976).
- Vergnaud Grazzini, C. et al. *Mar. Micropaleont.* **13**, 1–21 (1988).
- Devaux, M. thesis, Univ. Bordeaux (1985).
- Vergnaud Grazzini, C. *Science* **190**, 272–274 (1975).
- Vergnaud Grazzini, C. in *Geological Evolution of the Mediterranean Basin* (eds Stanley, D. J. & Wezel, F. C.) 413–451 (Springer, New York, 1985).
- Rotschy, F., Vergnaud Grazzini, C., Bellaiche, G. & Chamley, H. *Paleogeogr. Paleoclim. Paleocool.* **11**, 125–145 (1972).
- Paterne, M., Guichard, F., Labyrie, J., Gillot, P. & Duplessy, J. C. *Mar. Geol.* **72**, 259–285 (1986).
- Vergnaud Grazzini, C., Ryan, W. B. F. & Cita, M. B. *Mar. Micropaleont.* **2**, 353–370 (1977).
- Rossignol Strick, M., Nesteroff, W., Olive, P. & Vergnaud Grazzini, C. *Nature* **295**, 105–110 (1982).
- Buckley, H. A., Johnson, L. R., Shackleton, N. J. & Blow, R. A. *Deep Sea Res.* **29**, 739–766 (1982).
- Luz, B. *Nature* **278**, 847–848 (1979).
- Murat, A. thesis, Univ. Perpignan (1984).
- Sutherland, H. E., Calvert, S. E. & Morris, R. J. *Mar. Geol.* **56**, 79–92 (1984).
- Berger, W. H., Killingley, J. S. & Vincent, E. *Nature* **314**, 156–158 (1985).
- Duplessy, J. C. et al. *Nature* **320**, 350–352 (1986).
- Bard, E. et al. *Nature* **328**, 791–794 (1987).
- Labyrie, L., Duplessy, J. C. & Blank, P. *Nature* **327**, 477–482 (1987).
- Thiede, J. *Nature* **276**, 680–683 (1978).
- Thunell, R. C. *Quat. Res.* **11**, 353–372 (1979).
- Epstein, S., Buchsbaum, R., Lowenstam, H. A. & Urey, H. C. *Bull. geol. Soc. Am.* **64**, 1315–1326 (1953).
- Thunell, R. C., Williams, D. F. & Howell, M. *Paleoceanography* **2**, 661–678 (1987).
- Broecker, W. S. *Quat. Res.* **26**, 121–134 (1986).
- Street, F. A. & Grove, A. T. *Quat. Res.* **12**, 83–118 (1979).
- Hamilton, A. C. in *East African Vegetation* (eds Lind, E. & Morrison, M. E. S.) 188–209 (Longman, London, 1974).
- Zahn, R., Sarthein, M. & Erlenkeuser, H. *Paleoceanography* **2**, 543–559 (1987).
- Rossignol-Strick, M. *Nature* **304**, 46–49 (1983).
- Huang, T. C. & Stanley, D. J. *The Mediterranean Sea: A Natural Sediment Laboratory*, 512–559 (Dowden, Hutchinson and Ross, Stroudsburg, 1972).
- Aulsson, E. *Rep. Swed. Deep Sea Exped.* **8**, 353–391 (1961).
- Calvert, S. E. *Oceanol. Acta* **6**, 255–267.
- Howell, M. & Thunell, R. C. *Eos* **69**, 382 (1988).
- Maldonado, A. & Stanley, D. *Mar. Geol.* **31**, 215–250 (1979).

ACKNOWLEDGEMENTS. This research was partially supported by a grant from the NSF to R.C.T.

Probable modern analogue of Kuroko-type massive sulphide deposits in the Okinawa Trough back-arc basin

P. Halbach*, Ko-ichi Nakamura†, M. Wahsner*, J. Lange‡, H. Sakai§, L. Käselitz*, R.-D. Hansen‡, M. Yamano||, J. Post‡, B. Prause*, R. Seifert¶, W. Michaelis¶, F. Teichmann*, M. Kinoshita||, A. Märten*, J. Ishibashi§, S. Czerwinski* & N. Blum*

* Technical University of Clausthal, Institute of Mineralogy and Mineral Raw Materials, 3392 Clausthal-Zellerfeld, FRG

† Geological Survey of Japan, Marine Geology Department, Ibaraki, 305 Japan

‡ Preussag AG, 3000 Hannover, FRG

§ University of Tokyo, Ocean Research Institute, Tokyo 164, Japan

|| University of Tokyo, Earthquake Research Institute, Tokyo 113, Japan

¶ University of Hamburg, Geological and Paleontological Institute, 2000 Hamburg, FRG

* University of Karlsruhe, Institute for Petrology and Geochemistry, 7500 Karlsruhe, FRG

THE volcanogenic massive sulphide deposits of the Hokuroko district of Japan are Miocene metal occurrences which are rich in both Zn and Pb, contain minor Cu and yield noteworthy amounts of Ag and Au (ref. 1). They formed in the Green Tuff belt of Japan in association with the felsic calc-alkaline rocks of a back-arc spreading centre¹. Until now no modern analogue of this

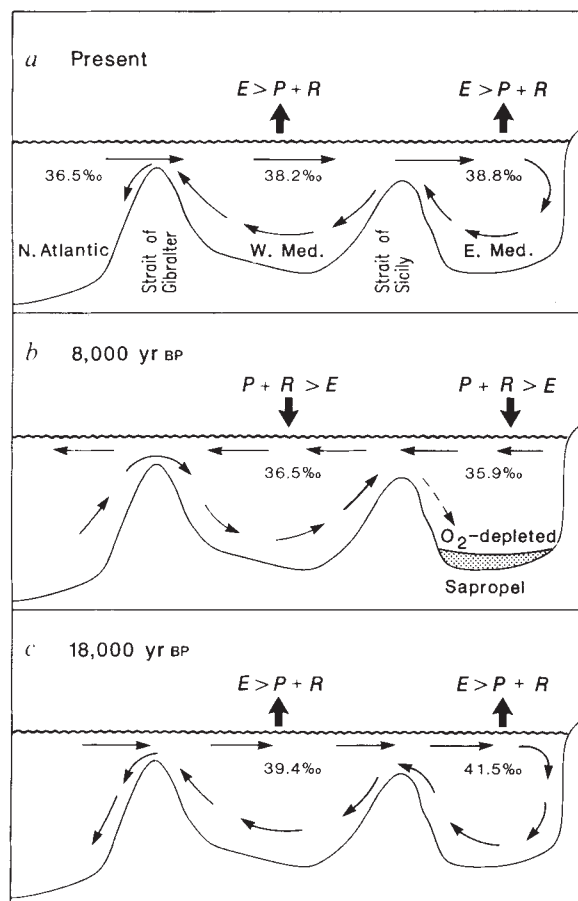


FIG. 2 Cross-sectional views of the Mediterranean showing estimated surface salinities, water balance and inferred circulation patterns for a the present; b, 8,000 yr BP, and c 18,000 yr BP. Here, E, P and R denote evaporation, precipitation and runoff.

'Kuroko' type of sulphide deposit has been identified; this is in contrast to the Cyprus type of massive Cu-Zn sulphide deposit, which has such an analogue in the 'smoker' deposits of the East Pacific Rise. In 1984 and 1986, low-temperature oxide and silicate precipitates were discovered in the rift valley of the Okinawa Trough attesting to the recent occurrence of hydrothermal activity in this intracontinental back-arc basin². On 26 June 1988, a large hydrothermal field with extensive occurrences of sulphide mineralization (the Jade hydrothermal field) was discovered and sampled by the German research vessel *Sonne* in a cauldron in the central part of the Okinawa Trough. Here we report geochemical and isotopic results which suggest that the Jade deposit is a modern analogue of the Kuroko-type deposits known so far only from the geological record.

The Okinawa Trough, located between the Ryukyu island chain and the east coast of China, is an active back-arc basin formed by extension within the continental lithosphere behind the Ryukyu trench-arc system. Evidence for the presence of active back-arc spreading exists only in the southern trough, whereas the main part of the graben system, characterized by normal faulting and crustal extension, is considered to be in the rifting phase³. The Middle Okinawa Trough probably represents a transition from present-day arc volcanism to volcanism associated with back-arc spreading. The local sea-floor map shows a series of parallel elongated ridges with 70° ENE strike, whereas the major structural grain is about 35° E which is indicative of a shear component in the deformation of the Middle Okinawa Trough. The ridges are composed of a bimodal assemblage of young volcanic rocks: basalt, andesite, dacite and rhyolite. First analyses made on board show that, according to their major-element geochemistry⁴, all the rock samples are of calc-alkaline island-arc type. The valleys and ridges are cut by normal faulting, giving rise to younger volcanic intrusions. Thus it is evident that the Okinawa Trough is a thermally active area^{5,6} in which magma supply systems and extension processes create hydrothermal circulation.

The Jade hydrothermal field was discovered in a caldera-like structure (the Izena Cauldron⁷) which has a diameter of about 5 km. It is located at about 27°15.0' N and 127°04.5' E and lies 36 km south of the deposit from the hydrothermal mounds (Fig. 1a). The central part of the cauldron, which has a maximum water depth of about 1,650 m, is filled with younger, unconsolidated sediments at least 10–20 m thick according to our 3.5-kHz record⁴. Preliminary results show that heat flow at the bottom of the cauldron is high and variable, which indicates that hydrothermal circulation is probably taking place. The measured heat flow ranges from 100 to 800 mW m⁻² and increases somewhat towards the north (Fig. 1b). By following this gradient, we discovered the hydrothermal vent area in the northern part of the inside north-east slope (Fig. 1b) by instrumented deep tow using the Ocean-Floor Observation System. Two photographic, temperature and television profiles were carried out across the vent area; temperature measurements showed anomalies of up to ~0.5 °C (Fig. 1b). The local topography of the Jade field is generally rugged with small step-fault features resulting from the intense tectonics within the inner cauldron slope. Animals typical of the hydrothermal-vent communities on the East Pacific Rise⁸, such as white clams, galatheid crabs and lugworms, as well as white starfish, are observed in our deep-sea pictures.

Distinct signals in the water column can be used to identify active hydrothermal-vent areas. Methane contents ranging from ~5.4 to ~6.2 p.p.m. were measured in water between 1,400 and 1,420 m above the hydrothermal field, at a total depth of 1,480 m. The Mn concentration measured above the vent area in the same depth range is ~2–3 parts per 10⁹ (p.p.b.) (measured background values of Mn varied between 0.2 and 0.3 p.p.b.). In the central part of the cauldron basin, concentrations as high as 8.8 p.p.m. CH₄ were measured at a depth of about 1,600 m; the CH₄ background in the surroundings of the cauldron is 0.02–0.05 p.p.m. These high CH₄ and Mn concentrations reveal

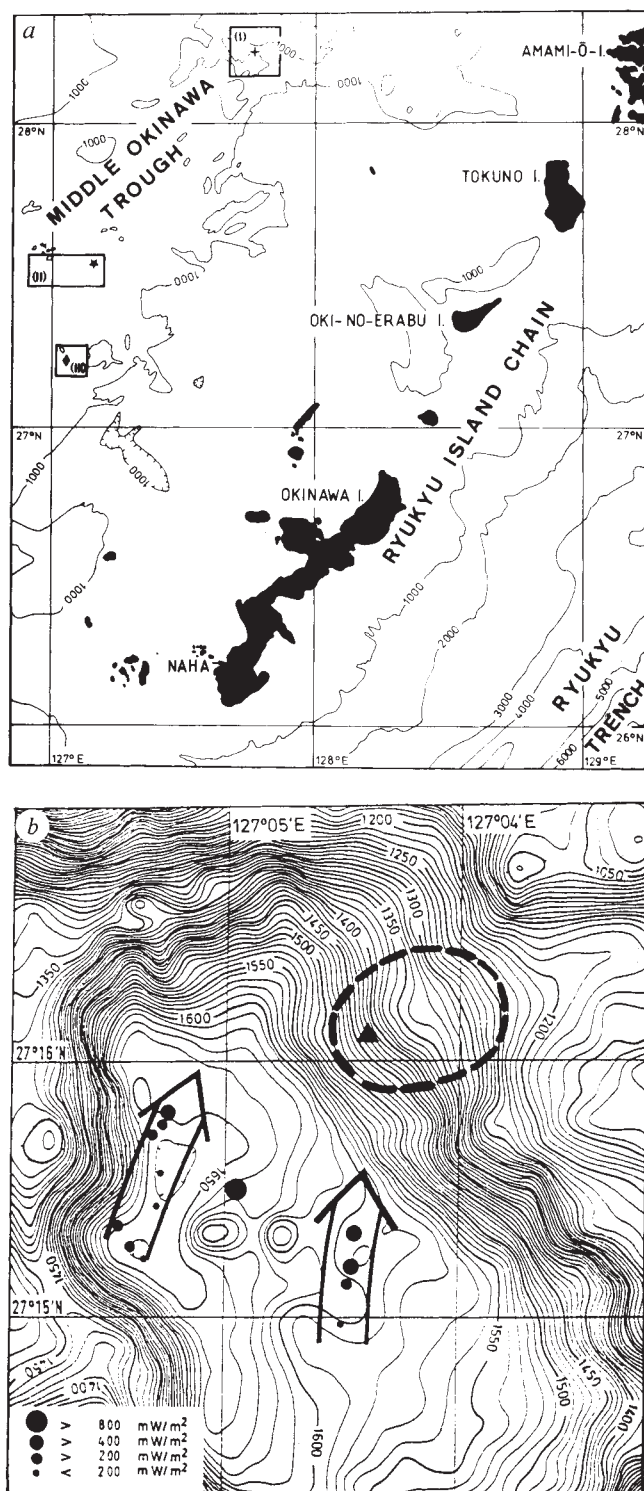


FIG. 1 Location maps for hydrothermal vents in the Okinawa Trough. a, Map¹⁵ of the central part of the Ryukyu trench-arc system, which is located between the island of Kyushu in the north-east and the island of Taiwan in the south-west. A section of the Middle Okinawa Trough is also shown. The areas (I), (II) and (III) were studied during the research cruise SO 56. + and * indicate locations of oxidic-siliceous low-temperature hydrothermal-mound deposits, ♦ shows the location of the Izena Cauldron with the Jade Hydrothermal Field. b, Part of the bathymetric map of the Izena Cauldron (location (III) in a). The Jade deposit lies on the inside north-east slope and is delimited by the dashed line. The contour interval of water depth is 10 m. ▲ marks the spot of the temperature anomaly (~0.5 °C) measured about 5 m above the bottom, which indicates hydrothermal venting. The two arrows show the approximate trend of increasing heat flow within the sediments of the basin, based on heat-flow data measured during the SO 56 cruise. The dots indicate heat-flow values.

the existence of a strong hydrothermal plume, which seems to be particularly pronounced because of the protected environment in the caldera.

We identified four different varieties of sea-bed sulphide mineralizations in the dredged material, namely two types of massive sulphides, a stockwork mineralization and a sulphide-bearing sediment layer. We now describe each of these in detail. (1) The first massive sulphide (MS I) comprised black-grey fragments of stacks, which accumulate around and on the hydrothermal vents by direct accretion of minerals precipitated from the hydrothermal fluid. Initial microscopic studies of the ore reveal that they represent a late stage of low-temperature hydrothermal activity. The youngest minerals in the outer layer (0–2 cm thick; Table 1, column A) are barite, realgar, orpiment, amorphous silica and millimetre-thick surface coatings of hydrous iron-manganese oxides. Other sulphide minerals are sphalerite (including schalenblende), Ag-bearing galena and pyrite (Table 1). The central parts (at 4–6 cm depth; Table 1, column C) consist mainly of the three sulphide minerals above; some chalcopyrite is present, but mostly as myrmekitic inclusions or as fine-grained replacement textures described as 'chalcopyrite disease'⁹. The barite and amorphous-silica content is significantly lower than in outer layers, and arsenic minerals are rarer.

(2) The second type of polymetallic sulphide (MS II) is olive-green to grey. The recovered material consists of elongated plates, several centimetres in thickness, which show a very fine and porous structure. The main minerals are sphalerite, chalcopyrite and pyrite, accompanied by anglesite and lesser amounts of amorphous silica. The higher concentration of chalcopyrite and the lack of galena probably indicates a higher primary temperature of formation relative to the MS I type. Anglesite was probably formed at higher oxygen fugacity than were the sulphides. Replacement of previously formed galena by anglesite was not observed.

(3) Within the host rocks of hydrothermally altered rhyolite are found millimetre-thick fracture fillings of stockwork mineralizations. These minerals are sphalerite, tennantite (Ag- and Sb-bearing), galena, enargite and, to a lesser extent, pyrite and chalcopyrite. The altered rhyolite consists of an extremely fine-

grained mixture of quartz, muscovite and some feldspar. Besides the fracture fillings, replacement masses are observed which contain basically the same sulphide minerals but with coarser grain-sizes. In some cracks of both the stockwork mineralization and the MS I type samples, millimetre-thick precipitates of native sulphur occur. Native sulphur is in general an oxidation product of H₂S. Its occurrence therefore indicates that in the venting hydrothermal fluid the concentration of H₂S was high and contributed to the formation of native sulphur after precipitation of the prevailing sulphide assemblage.

(4) Within two cores taken from the north-eastern part of the caldera basin, a sulphide-bearing layer of 10–15 cm in thickness was observed at a depth of about 80 cm below the top of the sediment. The fine-grained, unconsolidated, greyish material contains considerable amounts of sphalerite, pyrite and barite; the detrital minerals are quartz, calcite, illite and chlorite. As the sulphide-bearing sediment layer could not be observed in cores from the western part of the caldera basin, we suggest that the sulphide minerals were supplied from the Jade hydrothermal field.

Microthermometric data may be used to estimate mineral formation temperatures and salt concentrations of hydrothermal fluids. Such measurements have been conducted in the outer Ba- and As-rich material (sample A in Table 1) of the MS I type mineralizations. Each of the barite grains was found to be water-clear. The fluid inclusions have average lengths of 5–6 µm and are not bound to any cleavage. The nine measurements yielded fairly constant temperatures of homogenization of 160 ± 5 °C. The salt content of the fluid inclusions is somewhat higher than in normal ocean water (freezing temperature –1.9 °C), as indicated by freezing-point depressions ranging from –2.8 to –3.4 °C. Thus, this barite was formed at about 160 °C, probably through mixing of Ba-bearing hydrothermal solution with cold sulphate-rich sea water.

Chemical metal analyses (Table 1) indicate that the Jade massive sulphides are particularly rich in Zn and Pb but contain only minor amounts of Cu, with the exception of samples E (stockwork mineralization) and F (MS II type); samples A to D (MS I type) also exhibit significant Ag and Au content, with the Au/Ag ratio varying between 0.001 and 0.02, which is in the same range as values for other sea-floor massive sulphide deposits¹⁰. Point measurements, made by electron microprobe analysis, of galena grains in samples B and C revealed an Ag content of 0.28 ± 0.06% (32 measurements). Thus, in addition to tennantite, galena is an important host phase of Ag. No Ag was, however, detected in sphalerite.

In comparison with other recent sea-floor sulphide deposits^{10,11} the Ag concentration of 0.65% and the Au content of 9.8 p.p.m. (Table 1) are the highest found so far. Concentrations of Ba and As are found to be greatest in the upper 2 cm (Table 1, column A) of the MS I stack samples, indicating that barite, realgar and orpiment formed in the cooler and more oxidizing parts of the hydrothermal system. Au is related to these late precipitates; maximum concentrations of Ag occur more towards the inner parts (Table 1, columns B, C).

Au occurs at minor concentrations (0.3–0.7 p.p.m.; Table 1) in the chalcopyrite-rich samples of the MS II type and stockwork mineralizations, but is enriched by about one order of magnitude in the late paragenetic sulphide-sulphate-silica mineral system. Thus it is reasonable to assume that a remobilization of Au from the earlier, high-temperature sulphides has taken place, followed by reconcentration of Au in the more oxidized outer portions of the deposit through sustained hydrothermal circulation. Late, lower-temperature (<250 °C)¹¹ solutions ascending through massive sulphides would remove Au as the bisulphide complex (Au(HS)₂[–]) because of the high concentration of sulphur. Mixing with cooler sea water would result in a decrease in the solubility of Au, particularly below 200 °C (ref. 10). The chloride complex (AuCl₂[–]) is more important in early, high-temperature (300–400 °C) solutions¹⁰ and might account for the high Au content

TABLE 1 Metal composition of massive sulphide samples from the Jade Hydrothermal Field (Okinawa Trough)

	A*	B*	C*	D†	E‡	F§
Zn (%)	3.09	35.12	39.89	5.46	15.17	33.26
Cd	<0.01	0.06	0.11	<0.01	0.08	0.13
Pb	10.50	15.85	19.52	8.32	5.91	25.52
Fe	4.81	13.55	11.72	5.63	1.06	7.23
Mn	0.29	0.09	0.10	0.35	0.03	0.08
Cu	0.02	0.34	0.49	0.04	3.64	6.09
SiO ₂	19.36	7.56	1.04	20.63	49.20	2.53
Al	0.10	0.03	0.02	0.02	2.29	0.06
Ca	0.12	0.03	0.03	0.10	0.04	0.03
Mg	0.07	0.03	0.02	0.05	0.10	0.04
Sr	0.21	0.01	0.01	0.25	0.01	0.12
Ba	8.65	0.64	0.05	7.44	0.02	0.03
As	9.31	1.38	0.25	4.40	1.12	0.06
Ag	0.064	0.650	0.410	0.076	0.018	0.050
Au p.p.m.	7.8	9.8	4.8	4.2	0.3	0.7
Au/Ag	0.012	0.002	0.001	0.006	0.002	0.001

Metal determinations of dried substance were carried out by flameless atomic absorption spectrophotometry and inductive coupled plasma methods. All determinations were done twice; the relative deviation was less than 5%.

* Samples A–C correspond to MS I type: A, 0–2 cm; B, 2–4 cm; C, 4–6 cm.

† Surface sample from an MS I type stack fragment (comparable to sample A).

‡ Stockwork mineralization of hydrothermally altered rhyolite; analyses of bulk material taken from about 5 cm deeper than the oxidized surface layer.

§ Olive green MS II type sample.

in the more Cu-rich sulphide samples.

The sulphur isotope values $\delta^{34}\text{S}$ (% relative to CDT standard) of 17 pyrite samples from MS I type ores range from +4.3 to +10.7%, being generally between +6 and +8%. Values from barite lie within the range +21.9 to +23.6%. The Jade pyrites have substantially higher $\delta^{34}\text{S}$ values than other recent sea-floor sulphide deposits at mid-ocean ridges^{12,13}, being comparable with Kuroko sulphides, which have $\delta^{34}\text{S}$ values ranging from +5 to +8% (ref. 14). The Jade barites are isotopically similar to other barites, implying a seawater-sulphate origin ($\delta^{34}\text{S}$ values of modern sea water: +20.3%).

The Hokuroku district in northern Honshu is the type locality of the Kuroko massive sulphide occurrences, which are found in association with felsic calc-alkaline volcanic rocks¹. The latter consist of rhyolite and dacite, which intruded to shallow depths below the sea floor or poured out on the sea bottom. They generally underlie stratiform ore bodies, but may also serve as host rocks for stockwork mineralizations. In Kuroko deposits, Au-rich black ores are found stratigraphically above Au-poor, higher-temperature yellow ores and stockwork mineralizations¹¹. Our results suggest that the Jade hydrothermal field is analogous, in its nascent state, to the volcanogenic Kuroko-type massive sulphides. □

Received 12 December 1988; accepted 20 February 1989.

1. Ohmoto, H. & Skinner, B. J. *Econ. Geol. Monogr.* **5**, (1983).
2. Kimura, M. et al. *Tectonophysics* **145**, 319–324 (1988).
3. Sibuet, J. C. et al. *J. geophys. Res.* **92**, 14041–14063 (1987).
4. Halbach, P. et al. *Tech. cruise Rep. SO 56 (Hydromin-project)* prepared for the German Federal Ministry for Research and Technology, Clausthal-Zellerfeld (1989).
5. Yamano, M., Kinoshita, M. & Uyeda, S. *Proc. Int. Symp. on Geothermal Energy* Kumamoto-Beppu, Japan, 1–4 (1988).
6. Yamano, M., Uyeda, S. & Furukawa, Y. *Bull. Earthquake Res. Inst. Univ. Tokyo* **61**, 311–327 (1986).
7. Nakamura, K. et al. *Abstr. 4th Res. Symp. by the submersible "Shinkai 2000"* Tokyo, 5–6 (1987).
8. Hessler, R. R. & Smith, W. M. Jr in *Hydrothermal Processes at Seafloor Spreading Centres* (eds Rona, P. A., Bostrom, K., Laubier, L. & Smith, K. L.) 735–770 (Plenum, New York, 1983).
9. Barton, P. B. Jr *Min. Geol.* **28**, 293–300 (1978).
10. Hannington, M. D., Peter, J. M. & Scott, S. D. *Econ. Geol.* **81**, 1867–1883 (1986).
11. Hannington, M. D. & Scott, S. D. *Econ. Geol.* (submitted).
12. Arnold, M. & Sheppard, S. M. F. *Earth planet. Sci. Lett.* **56**, 148–156 (1981).
13. Shanks, W. C. & Seyfried, W. E. Jr *J. geophys. Res.* **92**, 11387–11399 (1987).
14. Kajiura, Y., Sasaki, A. & Matsubaya, O. *Geochem. J.* **15**, 193–197 (1981).
15. Oshima, S. et al. *Rep. Hydrogr. Res.* **24**, 19–43 (1988).

ACKNOWLEDGEMENTS. The cruise SO 56 was staged by the German–Japanese joint project to study hydrothermal activity in the Okinawa Trough. We thank the crew members and all scientists who participated. The research project was supported by a grant from the German Federal Ministry for Research and Technology. We thank Dr T. Moritani for his help, the Japanese Ministry for Foreign Affairs for the permission to work within the Japanese EEZ, and the Japanese Hydrographic Department for providing unpublished sea-floor maps. Dr Hein assisted in investigating the fluid inclusions and E. Riesen carried out part of the metal analyses. Dr F. Yanagisawa determined the $\delta^{34}\text{S}$ values reported here.

Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna

J. S. Singh, A. S. Raghubanshi, R. S. Singh & S. C. Srivastava

Department of Botany, Banaras Hindu University, Varanasi 221005, India

MORE than half of all tropical soils are highly weathered, leached and impoverished, requiring the ecosystem to develop nutrient-conserving mechanisms^{1,2}. Nutrient retention and withdrawal mechanisms are most effective in nutrient-poor systems^{3,4}. Thus, although dry tropical forests and savanna have the potential capacity to grow at high rates^{5,6}, this capacity is strictly limited by climate and nutrients. Our studies on these nutrient-poor ecosystems show that a reciprocal relationship exists between microbial biomass and plant growth rate. This suggests that microbial immobilization may be a main source of nutrients for the plants and may lead to nutrient conservation.

To study the factors affecting plant growth in nutrient-poor ecosystems, we selected sites in the Vindhyan hill tract (24°20'–25°10' N and 82°30'–83°30' E) of India. The soils at these sites are ultisols, derived from Kaimur sandstones (Dhandraul orthoquartzite). They are reddish brown in colour, sandy loam in texture^{6–7}, shallow, leached, and have moderate water-holding capacity (30–50%). The annual rainfall averages 800 mm, of which more than 75% is received during the monsoon season (late June to early September). November to February is a cool dry period (winter) and March to mid-June is hot and dry (summer). Although leaf emergence in most woody species precedes the rainy season, perhaps supported by nutrients withdrawn from senescing leaves, massive biomass accretion coincides with the growth spurt of the herbaceous layer triggered by the first showers of monsoon rain⁸. The herbs start drying up from October, leaving a substantial amount of their roots in the soil.

Every month five samples were collected from the upper 10-cm layers of soil at each site. Ammonium-N and nitrate-N were determined by the phenate and phenol-disulphonic acid methods, respectively, and phosphate-P was determined by the molybdate blue method^{9–11}. An *in situ* incubation technique was used to measure nitrification¹². Microbial C was determined using a modified liquid chloroform fumigation incubation technique, and microbial N and P were determined using fumigation extraction method^{13–15}.

The contents of extractable ammonium-N and phosphate-P were low and varied considerably within the annual cycle. In forest soils, nitrate-N varied from 0.4–5.6 μg per g dry soil, and in savanna soils from 0.4–0.8 μg per g. Values for ammonium-N were in the ranges 1.2–4.0 and 2.0–5.0 μg per g for forest and savanna sites, respectively. The contents of phosphate-P extracted using NaHCO_3 varied from 1.4–4.8 μg per g for forest soils and from 2.0–4.0 μg per g for savanna soils; the highest levels of phosphate-P as well as inorganic-N occurred in the summer. There was a marked seasonality in the rate of nitrification which peaked during the rainy season (Fig. 1); all soils showed little or no net nitrification during the dry period. The peak nitrate production rates in these ecosystems were lower than the minimum rates reported for tropical rain forest sites in Brazil, Costa Rica and Panama¹⁶. Low nutrient pools and low rates of nitrification show that these systems are extremely nutrient poor.

The highest and lowest levels of microbial biomass N occurred in the summer and rainy season, respectively, and the range of

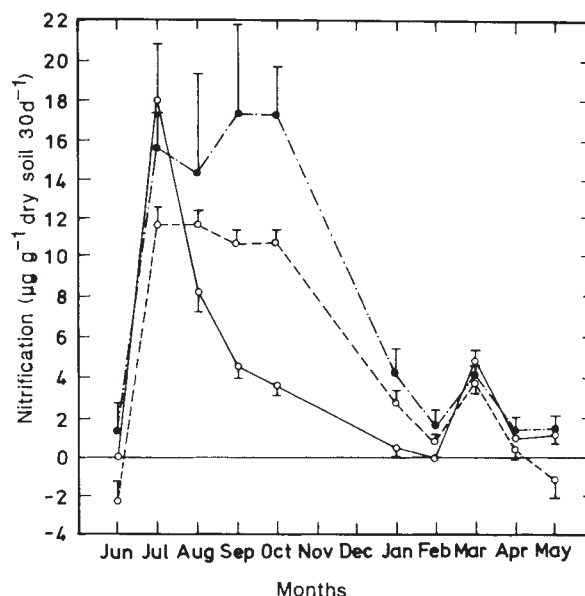


FIG. 1 Nitrification in dry tropical forest and savanna sites. ●—●, Forest; ○—○, grass savanna and ○—○, bamboo savanna (bars, 1 s.e.).

TABLE 1 Microbial biomass C, N and P in dry tropical ecosystems

Season	C	N	P
Forest			
Rainy	487 ± 9.2	51 ± 0.3	20 ± 1.7
Winter	662 ± 14.8	70 ± 1.2	29 ± 3.3
Summer	744 ± 10.3	88 ± 4.1	31 ± 1.6
Savanna			
Rainy	262 ± 7.5	31 ± 0.2	11 ± 1.5
Winter	400 ± 7.5	32 ± 0.7	19 ± 1.6
Summer	520 ± 9.4	46 ± 1.7	23 ± 1.8

Biomass C, N and P contents given as $\mu\text{g g}^{-1}$ dry soil. Differences due to seasons were significant at $P < 0.001$ for both the forest and savanna ecosystems.

values measured ($31\text{--}88 \mu\text{g per g}$) fell within that reported for the Amazonian rain forest sites¹⁷. Microbial immobilization of ^{15}N is reported to be greater at sites where the rates of nitrification are low than at sites where they are high¹⁶. Furthermore, the activity of soil microbes is less sensitive to soil water potential than is water uptake by the plants^{18–19}, and it is possible that a substantial amount of water is present at high tension during summer which is unavailable to plants but available to microbes¹. It seems, therefore, that microbial activity in these systems continues during dry months but that plant growth is drastically reduced, curtailing the demand for nutrients. Mineralization products and nutrients that move upward through capillary action in the dry season are thus immobilized in the microbial biomass.

In the ecosystems studied the microbial biomass and nutrient pools declined as N-mineralization increased, during the period when plant growth was most rapid. This correlation indicates that N immobilized in microbes is an important source of N for plants. This contrasts with the common assumption that steady-state mineralization is responsible for most of the nutrient supply to plants. Accelerated pulsed turnover of microbes in the rainy season may result from grazing by soil animals such as protozoa and nematodes. The populations of protozoa, fungivorous microarthropods and bacteria- and fungi-vorous nematodes expand in this season^{20–22}. Grazing of bacteria is necessary to make nutrients available for plant uptake^{23–24}. Mineral N is released mainly from dead microbial cells, microbial materials and microbial metabolites²⁵. Fresh plant material has to be partly humidified before mineral N might be released²⁶. The turnover of N from dead microbial cells is about five times that from native soil organic N^{27–28}. In low-N soils, microbial biomass is as effective as nitrate-N in supplying N to wheat²⁹. Vitousek and Matson³⁰ have concluded that microbial biomass, if conserved during forest management, retains N in harvested loblolly pine plantations.

We conclude that microbial biomass in these nutrient-poor systems acts as a sink and a source of nutrients. The principal function of the microbial biomass is thus to accumulate and conserve nutrients in a biologically active form during the dry period (high biomass, low turnover), when the activity of the plants is low and they are not able to extract nutrients effectively from soil, and then to release them rapidly during the monsoon period (low biomass, high turnover) to initiate plant growth. □

- Singh, V. K. & Singh, J. S. in *Environmental Degradation of Odra-Renukoot-Singrauli Area and its Impact on Natural and Derived Ecosystems* (ed. Singh, J. S.) 67–83 (Tech. Rep. Banaras Hindu Univ., 1988).
- Methods of Soil Analysis* (ed. Black, C. A.) (Am. Soc. Agron., Monogr. 9, Pt I, Madison, Wisconsin, 1965).
- Jackson, M. L. *Soil Chemical Analysis* (Constable, London, 1958).
- Standard Methods for the Examination of Water and Wastewater* (Am. Pub. Health Ass., Washington, 1985).
- Eno, C. F. *Soil Sci. Soc. Proc. Am.* **24**, 277–279 (1960).
- Srivastava, S. C. & Singh, J. S. *Soil Biol. Biochem.* **20**, 743–747 (1988).
- Jenkinson, D. S. & Powelson, D. S. *Soil Biol. Biochem.* **8**, 209–213 (1976).
- Brookes, P. C., Powelson, D. S. & Jenkinson, D. S. *Soil Biol. Biochem.* **14**, 319–329 (1982).
- Vitousek, P. M. & Matson, P. A. *Soil Biol. Biochem.* **20**, 361–367 (1988).
- Livingston, G. P., Vitousek, P. M. & Matson, P. A. *J. geophys. Res.* **93** (D3), 1593–1599 (1988).
- Calder, E. A. *J. Soil. Sci.* **8**, 60–72 (1957).
- Semb, G. & Robinson, J. B. D. *East Afr. Agr. J.* **34**, 350–370 (1969).
- Dash, M. C. & Guru, B. C. *Pedobiologia* **20**, 325–342 (1980).
- Singh, J. & Mukherjee, I. N. *J. Soil Biol. Ecol.* **6**, 104–108 (1986).
- Dwivedi, B. K., Kumar, A., Shukla, R. C. & Misra, S. L. *J. Soil Biol. Ecol.* **7**, 90–97 (1987).
- Clarholm, M. *Soil Biol. Biochem.* **17**, 181–187 (1985).
- Coleman, D. C. *et al.* *Soil Organisms as Components of Ecosystems* (eds Lohm, U. & Persson, T.) 299–309 (Ecol. Bull., Stockholm, 1977).
- Cameron, R. S. & Posner, A. M. *J. Soil Sci.* **30**, 565–577 (1979).
- Bernhard-Reversat, F. *Oikos* **38**, 321–332 (1982).
- Stevenson, F. J. *Cycles of Soil* (Wiley, New York, 1986).
- Marumoto, T., Kai, H., Yoshida, T. & Harada, T. *Soil Sci. Pl. Nutr.* **23**, 125–134 (1977).
- Lathbridge, G. & Davidson, M. S. *Soil Biol. Biochem.* **15**, 375–376 (1983).
- Vitousek, P. M. & Matson, P. A. *Science* **225**, 51–52 (1984).

ACKNOWLEDGEMENTS. This study was supported by the University Grants Commission and the Ministry of Environment and Forests, New Delhi.

Temporally distinct pre- and post-synaptic mechanisms maintain long-term potentiation

Stephen N. Davies, Robin A. J. Lester*, Klaus G. Reymann† & Graham L. Collingridge

Department of Pharmacology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK

LONG-TERM potentiation (LTP) in the hippocampus is widely studied as the mechanisms involved in its induction and maintenance are believed to underlie fundamental properties of learning and memory in vertebrates¹. Most synapses that exhibit LTP use an excitatory amino-acid neurotransmitter that acts on two types of receptor, the N-methyl-D-aspartate (NMDA) and quisqualate receptors². The quisqualate receptor mediates the fast synaptic response evoked by low-frequency stimulation^{3,4}, whereas the NMDA receptor system is activated transiently by tetanic stimulation, leading to the induction of LTP^{3,5–7}. The events responsible for maintaining LTP once it is established are not known. We now demonstrate that the sensitivity of CA1 neurons in hippocampal slices to ionophoretically-applied quisqualate receptor ligands slowly increases following the induction of LTP. This provides direct evidence for a functional post-synaptic change and suggests that pre-synaptic mechanisms also contribute, but in a temporally distinct manner, to the maintenance of LTP.

The excitatory post-synaptic potential (e.p.s.p.) evoked in CA1 neurons by low frequency stimulation of the Schaffer collateral-commissural pathway (see Fig. 1a) is depressed by the quisqualate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)^{4,8–10}. In physiological medium, NMDA antagonists do not affect this e.p.s.p. either before or after the induction of LTP³; it is therefore assumed that the potentiated e.p.s.p. is mediated by quisqualate receptors. To test this directly, we compared the sensitivity to CNQX of potentiated and unpotentiated inputs onto the same population of CA1 neurons. As illustrated in Fig. 1, $10 \mu\text{M}$ CNQX, a concentration that depresses responses of CA1 neurons to quisqualate and AMPA

* Present address: Vollum Institute for Advanced Biomedical Research, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97201, USA.

† Permanent address: Institute of Neurobiology and Brain Research, Academy of Sciences GDR, Leipzigerstrasse 44, DDR-3090 Magdeburg, GDR.

Received 5 December 1988; accepted 13 February 1989.

- Sanchez, P. A. *Properties and Management of Soils in the Tropics* (Wiley, New York, 1976).
- Jordan, C. F. *Nutrient Cycling in Tropical Forest Ecosystems* (Wiley, Chichester, 1985).
- Chapin III, F. S. *A. Rev. Ecol. Syst.* **11**, 233–263 (1980).
- Singh, J. S., Rawat, Y. S. & Chaturvedi, O. P. *Nature* **311**, 54–56 (1984).
- Singh, J. S. & Yadava, P. S. *Ecol. Monogr.* **44**, 351–376 (1974).
- Singh, K. P. & Misra, R. *Structure and Functioning of Natural, Modified and Silvicultural Ecosystems of Eastern Uttar Pradesh* (Tech. Rep., Banaras Hindu Univ., 1979).
- Benchmark Soils of India* (eds Murthy, R. S., Hirekerur, L. R., Deshpande, S. B. & Venkata Rao, B. V.) (National Bureau of Soil Survey and Landuse Planning (ICAR), Nagpur, 1982).

but not NMDA⁴, depressed both inputs in parallel ($n = 6$). Thus, the receptors that mediate the potentiated response are probably of the quisqualate receptor subtype. This result means that if LTP is maintained by post-synaptic mechanisms there should be an associated increase in the sensitivity of neurons to appropriately administered quisqualate receptor ligands.

To test for this, intracellular recordings were obtained from CA1 pyramidal neurons (Fig. 2). In five cells, tetanic stimulation elicited immediate and stable LTP; the respective mean ($\pm$ s.e.m.) amplitudes of the e.p.s.p., measured from averages of 10 successive responses, 0–5 min before and 25–30 min after the induction of LTP were 5.3 ± 0.8 and 8.5 ± 1.3 mV (mean increase, $53 \pm 12\%$). The generation of LTP was not associated with any significant change in either the resting membrane potential or resting input resistance; the values at the corresponding times were -69 ± 2 and -70 ± 2 mV, and 29 ± 2 and 31 ± 3 M Ω , respectively. In these cells, quisqualate elicited depolarizations of 5.7 ± 0.7 mV, measured from averages of 10 successive responses 0–5 min before the induction of LTP. By contrast with the synaptic response, quisqualate-induced depolarizations were not immediately affected by tetanic stimulation. For example, the mean depolarization 5–10 min after the induction of LTP was still 5.7 ± 0.7 mV. Thereafter, however, there was a tendency for the quisqualate-induced responses to increase in size. In four cells there was an increase ($21 \pm 6\%$) by 25–30 min post-tetanus and in the fifth cell the response size showed a comparable increase by 60 min. By contrast, in three cells tetanic stimulation resulted in neither the generation of LTP nor an increase in sensitivity to quisqualate. Thus, in only those cells where stable LTP had been induced was there a delayed increase in sensitivity to quisqualate.

To quantify the effect further, stable extracellular d.c. potentials¹¹ were obtained in response to ionophoretic administration of the selective quisqualate receptor agonist AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) at 15-min intervals for 1 h before and 3 h following tetanic stimulation (Fig. 3). In control medium this elicited LTP in 9 out of 11 slices;

the mean change in the field e.p.s.p. amplitude (and slope) 2 h after the tetanus for all 11 slices was $+47 \pm 21\%$ ($+82 \pm 34\%$) of control. Usually the sensitivity of the cells to the first post-tetanus AMPA application was unaltered. In most cases, however, the sensitivity to AMPA then gradually increased, reaching a maximum after about 2 h and persisting throughout the 3-h period following the tetanus. The mean change for all 11 slices at 2 h was $+62 \pm 25\%$ ($P < 0.05$; paired t -test).

If the change in AMPA sensitivity is the result of the generation of LTP, it should be blocked by treatments that prevent the induction of LTP, such as adding an NMDA antagonist³. In the presence of $50 \mu\text{M}$ D-2-amino-5-phosphonovalerate (APV) there was neither the production of LTP (mean change in field e.p.s.p. 2 h after tetanus was $-9 \pm 9\%$ ($-2 \pm 8\%$) of control), nor any consistent change in the sensitivity of CA1 neurons to AMPA (Fig. 3). The mean change in the amplitude of the AMPA responses 2 h post-tetanus was $-7 \pm 12\%$ ($P > 0.05$; paired t -test). In three slices, tetanic stimulation was delivered first in the presence of APV and, 3 h later, after washout of APV; invariably APV reversibly prevented both the induction of LTP and the sensitivity change to AMPA (Fig. 3).

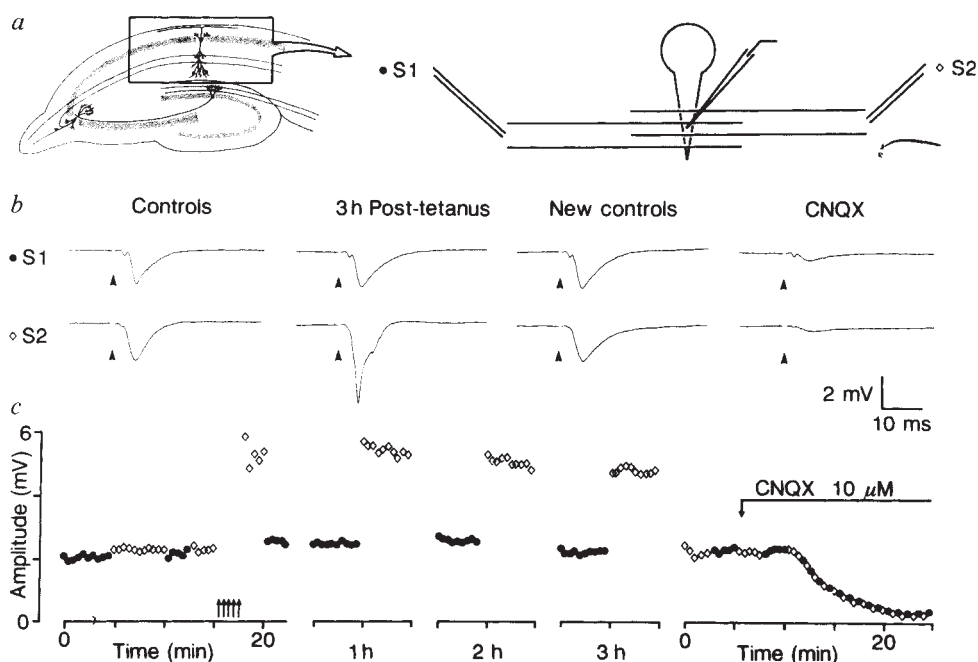
To examine whether the potentiated AMPA response involved activation of local synaptic circuitry, we tested the effects of a Ca^{2+} -channel antagonist on responses 2 h after tetanization. In these slices, synaptic and AMPA-induced responses had increased 2 h post-tetanus by $33 \pm 7\%$ ($62 \pm 15\%$) and $43 \pm 8\%$ ($n = 4$), respectively. Cadmium (50 – $100 \mu\text{M}$) reduced the amplitude of the synaptic response by 56 – 70% but had no effect on the amplitude of the AMPA-evoked depolarizations.

Whether LTP is maintained by pre-synaptic^{12–14} or post-synaptic^{15–17} mechanisms is controversial. Our data suggest that in the Schaffer collateral-commissural pathway both mechanisms occur but with different time courses. Previous studies have failed to detect an increase in sensitivity to ionophoretically administered agonists with LTP^{18–20}. There are two explanations for this. First, the sensitivity to exogenously applied agonists was followed only for short periods and, as we show, sensitivity

FIG. 1 CNQX blocks in parallel potentiated and unpotentiated field e.p.s.p.s.

a, Diagrammatic representation of the recording arrangement for this series of experiments; an extracellular recording electrode was placed in stratum radiatum of area CA1 and two stimulating electrodes (S1 and S2) were positioned ~ 1 mm on either side. A large separation was used to minimize the extent to which both electrodes stimulated the same fibres. LTP was induced by stimulating one input using five trains delivered at 30-s intervals. Each train comprised 100-Hz stimulation for 1 s at double test intensity. After the induction of LTP (15, 30 or 180 min) the amplitude of the two inputs was matched by increasing the stimulus intensity of the control input or by decreasing that of the potentiated input and CNQX was then applied. **b**, An example from one slice of field e.p.s.p.s. evoked by the two stimulating electrodes showing from left to right, matched control responses (2.8 and 3.0 V), responses after tetanization of S2, new matched controls after S2 intensity had been reduced to 2.1 V, and finally the effect of CNQX ($10 \mu\text{M}$) on the two responses. In these and subsequent figures synaptic records are averages of 3–10 consecutive responses and the position of the blanked stimulus artefacts are indicated by arrowheads.

c, Amplitude of the field e.p.s.p.s. plotted throughout the experiment illustrated in **b** showing the parallel reduction by CNQX of the potentiated and the unpotentiated response.



METHODS. Rat hippocampal slices ($400\text{-}\mu\text{m}$ thick) were prepared and maintained in an interface type chamber at 30 – 32°C , using standard techniques³. They were perfused with medium containing: NaCl, 124 mM; KCl, 3 mM; NaHCO_3 , 26 mM; CaCl_2 , 2 mM; MgSO_4 , 1 mM; D-glucose, 10 mM; NaH_2PO_4 , 1.25 mM (omitted for cadmium experiments), bubbled with a 95% $\text{O}_2/5\%$ CO_2 mixture.

changes are usually slow to develop. Second, L-glutamate was used which, unlike AMPA and quisqualate in hippocampal slices⁴, exerts only part of its action through CNQX-sensitive receptors²¹. Our results favour a pre-synaptic component to the early maintenance of LTP in the Schaffer collateral-commissural pathway because LTP was produced within 30 s, yet sensitivity changes were not usually detected for at least 15 min and took ~2 h to reach a maximum.

An intriguing feature of the post-synaptic change is its slow development. Interestingly, in the presence of protein kinase C (PKC) inhibitors, LTP lasts for only 1–2 h (refs 22–24); a time similar to that required for maximal potentiation of the AMPA-induced responses. Moreover, injection of purified PKC into the post-synaptic neuron induces an LTP-like change in synaptic transmission²⁵. It is possible that PKC contributes to the initiation of this slowly developing, postsynaptically-located com-

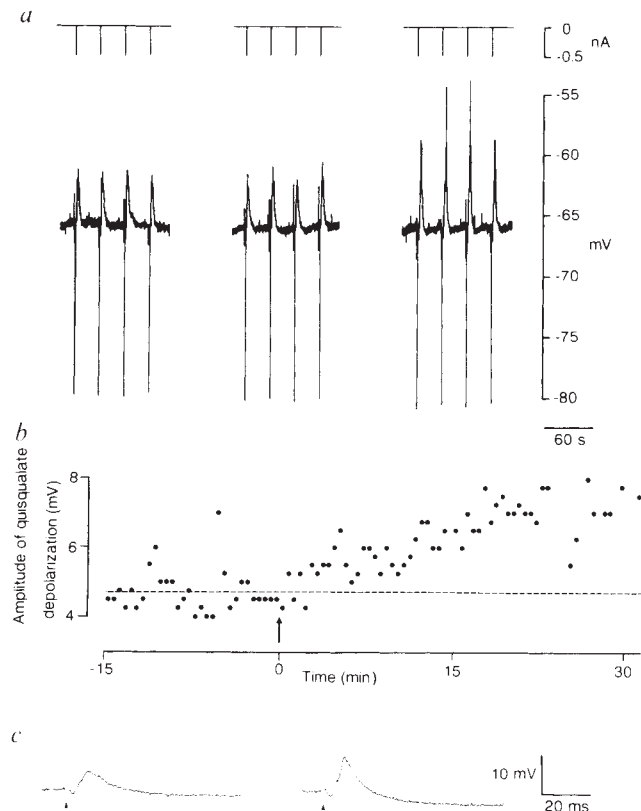
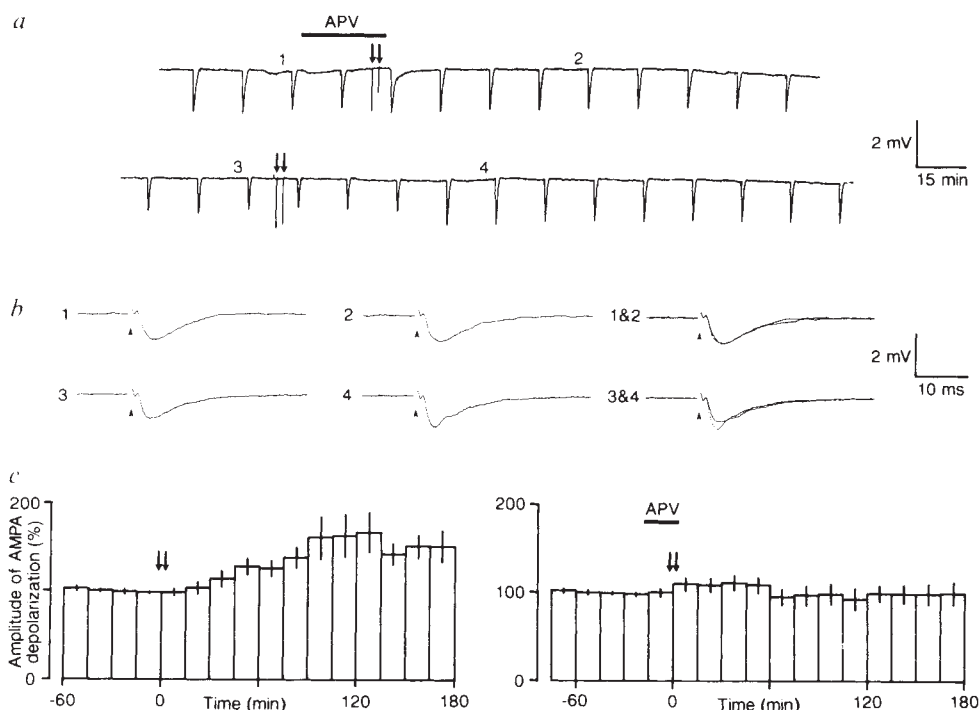


FIG. 2 LTP is associated with a delayed increase in sensitivity of single CA1 neurons to quisqualate. *a*, Current (top) and voltage (bottom) records from an intracellularly recorded CA1 pyramidal neuron. Depolarizations are in response to ionophoretic ejection of quisqualate (130 nA, 2 s) into stratum radiatum. These are preceded by hyperpolarizations in response to constant current pulses (0.5 nA, 400 ms), used to monitor resting input resistance. Panels illustrate from left to right, control records, sections taken immediately after, and sections taken 30 min after the tetanus (100 Hz, 1 s, test intensity). Two of the last four quisqualate-induced responses depolarize the cell sufficiently to fire action potentials. *b*, Plot showing the amplitude of quisqualate-induced depolarizations for 15 min before and 30 min following the tetanus (arrow). Thereafter (for a further 45 min) quisqualate-induced depolarizations excited the cell sufficiently to elicit firing. *c*, Synaptic responses taken immediately before and 30 min after the tetanus.

METHODS. Intracellular recordings were obtained from the cell-body layer using electrodes containing 3 M potassium acetate and the Schaffer collateral-commissural pathway was stimulated at 30 s intervals by a single electrode placed in stratum radiatum. An ionophoretic pipette was positioned in stratum radiatum at the level of the stimulated fibres, adjacent to the recording site. Quisqualate (20 mM in 150 mM NaCl) was applied ionophoretically (46–163 nA, 1–2 s) 5 s after each synaptic response was elicited. The position of the ionophoretic pipette was adjusted to achieve a compromise between short latency responses (indicating that the pipette was close to a dendritic branch of the recorded cell) and the maintenance of stable recordings. Only those cells where passive membrane properties and both synaptic and agonist-induced responses remained stable for 15 min before tetanic stimulation (100 Hz, 1 s, test intensity) and where healthy impalements could be maintained for at least a further 30 min were included in the analysis.

FIG. 3 The delayed increase in sensitivity is dependent on the synaptic activation of NMDA receptors. *a*, d.c. potentials evoked in the stratum radiatum by ionophoretic ejection of AMPA (87 nA, 20 s) every 15 min. In the upper panel APV (50 μ M) was present during the tetani and there was minimal change in the size of the AMPA-induced responses. In the lower panel a second pair of tetani delivered 3 h later in the absence of APV induced a delayed increase in the amplitude of the AMPA-induced responses. The two sections of record are continuous. *b*, Field e.p.s.p.s recorded at times indicated, showing synaptic responses before and 1 h after the tetani. *c*, Pooled data showing effect of tetanization on amplitude of AMPA responses (normalized to mean of control responses) in the absence ($n=11$) and presence ($n=8$) of APV (50 μ M). Error bars show ± 1 s.e.m.

METHODS. The experimental arrangement was similar to that shown in Fig. 1, except that the two stimulating electrodes were placed closer together to maximize the number of tetanized synapses in the vicinity of the recording electrode. Each pathway was tetanized once (100 Hz, 1 s, double test intensity). AMPA (20 mM in 150 mM NaCl) was applied ionophoretically



(40–155 nA, 10–25 s, at 15 min intervals) from a pipette close to the recording electrode.

ponent of LTP. The early presynaptic component presumably involves PKC-independent mechanisms. One possibility is that NMDA receptor activation releases arachidonic acid metabolites²⁶ from the post-synaptic cell and that these act as retrograde messengers which result in the enhanced neurotransmitter release^{27,28}. □

Received 5 January; accepted 23 February 1989.

- Bliss, T. V. P. & Lynch, M. A. *Neurol. Neurobiol.* **35**, 3–72 (1988).
- Watkins, J. C. & Evans, R. H. A. *Rev. Pharmac. Tox.* **21**, 165–204 (1981).
- Collingridge, G. L., Kehl, S. J. & McLennan, H. J. *Physiol., Lond.* **334**, 33–46 (1983).
- Blake, J. F., Brown, M. W. & Collingridge, G. L. *Neurosci. Lett.* **89**, 182–186 (1988).
- Harris, E. W., Ganong, A. H. & Cotman, C. W. *Brain Res.* **323**, 132–137 (1984).
- Wigström, H. & Gustafsson, B. *Neurosci. Lett.* **44**, 327–332 (1984).
- Herron, C. E., Lester, R. A. J., Coan, E. J. & Collingridge, G. L. *Nature* **322**, 265–268 (1986).
- Honoré, T. *et al. Science* **241**, 701–703 (1988).
- Andreasen, M., Lambert, J. D. C. & Skovgaard Jensen, M. *Neurosci. Lett.* **93**, 61–66 (1988).
- Neuman, R. S., Ben-Ari, Y., Gho, M. & Cherubini, E. *Neurosci. Lett.* **92**, 64–68 (1988).
- Lambert, J. D. C., Flatman, J. A. & Jahnsen, H. J. *Neurosci. Meth.* **3**, 311–315 (1981).
- Bliss, T. V. P., Douglas, R. M., Errington, M. L. & Lynch, M. A. *J. Physiol., Lond.* **377**, 391–408 (1986).
- Skrede, K. & Malthé-Sørensen, D. *Brain Res.* **208**, 436–441 (1981).
- Sastry, B. R. *Life Sci.* **30**, 2003–2008 (1982).
- Lynch, G. & Baudry, M. *Science* **224**, 1057–1063 (1984).
- Kauer, J. A., Malenka, R. C. & Nicoll, R. A. *Neuron* **1**, 911–917 (1988).
- Muller, D., Joly, M. & Lynch, G. *Science* **242**, 1694–1697 (1988).
- Lynch, G., Gribkoff, V. & Deadwyler, S. A. *Nature* **263**, 151–153 (1976).
- Mohan, P. M. & Sastry, B. R. *Eur. J. Pharmac.* **114**, 335–341 (1985).
- Taube, J. S. & Schwartzkroin, P. A. *J. Neuroscience* **8**, 1632–1644 (1988).
- Davies, S. N., Fletcher, E. J. & Lodge, D. J. *Physiol., Lond.* **406**, 13P (1988).
- Lovinger, D. M., Wong, K. L., Murakami, K. & Routtenberg, A. *Brain Res.* **436**, 177–183 (1987).
- Reymann, K. G., Frey, U., Jork, R. & Matthies, H. *Brain Res.* **440**, 305–314 (1988).
- Malinow, R., Madison, D. V. & Tsien, R. W. *Nature* **335**, 821–824 (1988).
- Hu, G.-Y. *et al. Nature* **328**, 426–429 (1987).
- Dumuis, A., Sebben, M., Haynes, L., Pin, J.-P. & Bockaert, J. *Nature* **336**, 68–70 (1988).
- Piomelli, D. *et al. Nature* **328**, 38–43 (1987).
- Williams, J. H. & Bliss, T. V. P. *Neurosci. Lett.* **88**, 81–85 (1988).

ACKNOWLEDGEMENTS. APV and AMPA were gifts from Dr J. C. Watkins and CNQX from Dr T. Honoré. We thank Dr R. H. Evans for the loan of the ionophoretic equipment which was provided by a grant from the Royal Society. This work was supported by the MRC and by travel grants to K.G.R. from the Wellcome Trust and the Academy of Sciences GDR.

Presentation of antigen, foreign major histocompatibility complex proteins and self by thymus cortical epithelium

Philippa Marrack*†‡, James McCormack & John Kappler†‡

Howard Hughes Medical Institute, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, and Departments of Biochemistry, Biophysics and Genetics*, and Microbiology and Immunology†, and Medicine‡, University of Colorado, Denver, Colorado 80207, USA

IN mouse and man most peripheral T cells bear clonally variable receptors made up of α - and β -chains¹ which bind ligands on target cells consisting of peptide fragments of foreign antigens, complexed with cell surface proteins encoded by the major histocompatibility complex (MHC) of the individual^{2–4}. In the thymus, developing T cells are selected to mature only if their receptors will be able to participate in self-MHC plus antigen recognition in the periphery^{5–8}. This positive selection occurs in the presence of self-MHC, but in the apparent absence of antigen, leading to the paradoxical conclusion that developing thymocytes must be positively selected by engagement of their receptors and self-MHC alone, although thymocytes that react too well with self-MHC are eliminated⁹. To account for this, it has been suggested that MHC molecules in the thymus are not identical to those found elsewhere¹. To test this and other hypotheses, we have examined the ability of the presumed selecting cells, those of the thymus cortical epithelium^{10,11}, to present various MHC complexes to T cells. Our results indicate that MHC molecules on thymus epithelium are not always the same as those found elsewhere.

Thymus cortical epithelial cells were isolated as nurse cells¹² from BALB/c (H-2^d) mice and tested for their ability to present antigen plus I-A^d, or I-E^d to T-cell hybridomas. T-cell-depleted BALB/c spleen cells were used as controls. As shown in Fig. 1a and b, a T-cell hybridoma specific for I-E^d as an alloantigen, CG4, responded equally well to the nurse cell and spleen cell preparations in the dose ranges tested. Similar results were obtained using a T-cell hybridoma from BALB/c mice, specific for a peptide of chicken ovalbumen (cOVA)/I-A^d, 3DO-8.2 (Fig. 1c, d). Kyewski *et al.*¹³ have previously shown that thymus epithelial cells cannot present antigen to T-cell clones. The discrepancy between their results and those shown here may be due to our use of T-cell hybridomas, cells which, unlike the normal T cells used by Kyewski *et al.*, require only α/β -receptor engagement and no secondary signals to respond.

Our results show that some cells in the thymus epithelium preparation can present allogeneic MHC or antigen plus self-MHC to T-cell hybridomas. Although the active cells could be contaminant macrophages or dendritic cells, it seems that the epithelial cells themselves contribute to the presentation: not only did the nurse-cell preparations contain no or few rosette-forming dendritic cells or macrophages, but their antigen-presenting activity did not fall off upon selectively enriching for nurse cells (data not shown). Also, as shown below, the nurse cell preparations were selectively deficient in their ability to present antigen-MHC complexes which are expressed on B cells and macrophages *in vivo*.

Recently it has been shown that almost all T-cell receptors that include V β 17a impose reactivity for IE plus a B-cell-derived product on T cells which bear them^{14,15}. Despite this, V β 17a⁺ cells seem to react with I-E on macrophages isolated from mice, perhaps because of transfer of the B-cell-derived product from one cell to another *in vivo*. Indirect evidence, however, suggested that the V β 17a ligand might not exist on selecting cells in the thymus, as V β 17a⁺ cells are not 'overselected' in thymuses which express I-E on thymus epithelium, but elsewhere¹⁶. To test this, three V β 17a⁺ T-cell hybridomas were challenged with I-E^d on BALB/c nurse or spleen cells. The hybridomas responded poorly to nurse cells, and much better to spleen cells (Fig. 1e, f). This indicates that V β 17a⁺ T cells do not react well, if at all, with IE on thymus nurse cells, as the small responses seen against our epithelial cell preparations may have been against contaminating cells. Preliminary experiments show that nurse cells from transgenic mice, expressing I-E on only thymus epithelial cells, do stimulate weak responses from some V β 17a⁺ hybrids, indicating that the V β 17a ligand might be present on thymus epithelium, albeit at low levels.

We also tested the ability of T-cell hybrids to react with syngeneic thymus epithelium in the absence of antigen. A BALB/c-derived T-cell hybridoma specific for cOVA/I-A^d, 3DO-54.8, reacted with the BALB/c nurse cell preparation in the absence of antigen (Fig. 1g, h). This T cell did not react with spleen cells in the absence of cOVA, even when the spleen cells had been subjected to the same enzyme treatments as the nurse cells, nor did it react with BALB/c-derived, I-A^d-bearing macrophages or B-cell lymphomas. The failure of 3DO-54.8 to react with spleen cells was not due to a splenic inhibitory activity, as mixtures of splenic and nurse cells stimulated this and another hybrid as well as the nurse cell preparations alone (data not shown).

Several independent T-cell hybridomas were tested for their ability to react with syngeneic nurse cells (Fig. 2). The BALB.B (H-2^b)-derived hybridomas, BO-97.10 and 2BO-43 responded quite well to H-2^b-bearing nurse cell; BO-97.10 responded only slightly to spleen cells from the same animals. These hybridomas did not react significantly with H-2^d nurse or spleen cells. A third H-2^b-derived hybridoma, 2BO-33, did not react with any preparation, and two H-2^d-derived hybridomas, 22DO-14 and 3DO-54.8, reacted with H-2^d nurse but not H-2^d spleen cells. The hybridoma 3DO-54.8 failed to react with the H-2^b cell

preparations, and 22DO-14 reacted only slightly with both nurse and spleen cell preparations from the H-2^b mice. Of 35 T-cell hybridomas screened, at least 54% react to some degree with nurse cells from syngeneic mice. By contrast, 26% respond to syngeneic spleen cells, or allogeneic nurse or spleen cells. Usually the reactivities to syngeneic nurse cells are small by comparison with responses to specific antigen plus MHC, or anti-receptor antibody, by the same hybrids. We have been unable to enhance these reactivities by increasing the incubation times of the nurse cells with the T cells, or by adding gamma interferon or indomethacin.

Many of the hybridomas that responded to syngeneic nurse cells were produced by fusion of normal T cells to a derivative of the thymoma, BW 5147 (ref. 17), which does not express functional T-cell receptor α - and β -chain messenger RNA. The only T-cell receptors expressed on these cells which could be involved in reaction with the syngeneic thymus epithelial cells were therefore those derived from their normal T-cell parents. The hybridoma 3DO-54.8, however, was produced by fusion of a normal T cell to BW5147, which itself contains functional α - and β -chain mRNA; it may thus express up to four different receptors on its surface, by combination of the normal T-cell and BW receptor polypeptides.

To show that the BALB/c-selected cOVA/I-A^d receptor on 3DO-54.8 was responsible for the reactivity of this hybrid with syngeneic nurse cells, we tested it and several of its derivatives under various conditions for their ability to respond to BALB/c nurse cells. As before, 3DO-54.8 responded to the nurse cells; this response was inhibited by antibodies to the restricting MHC molecule for 3DO-54.8, I-A^d. A subclone of the hybridoma, 3DO-54.8.21, in which the β -chain gene inherited from its nor-

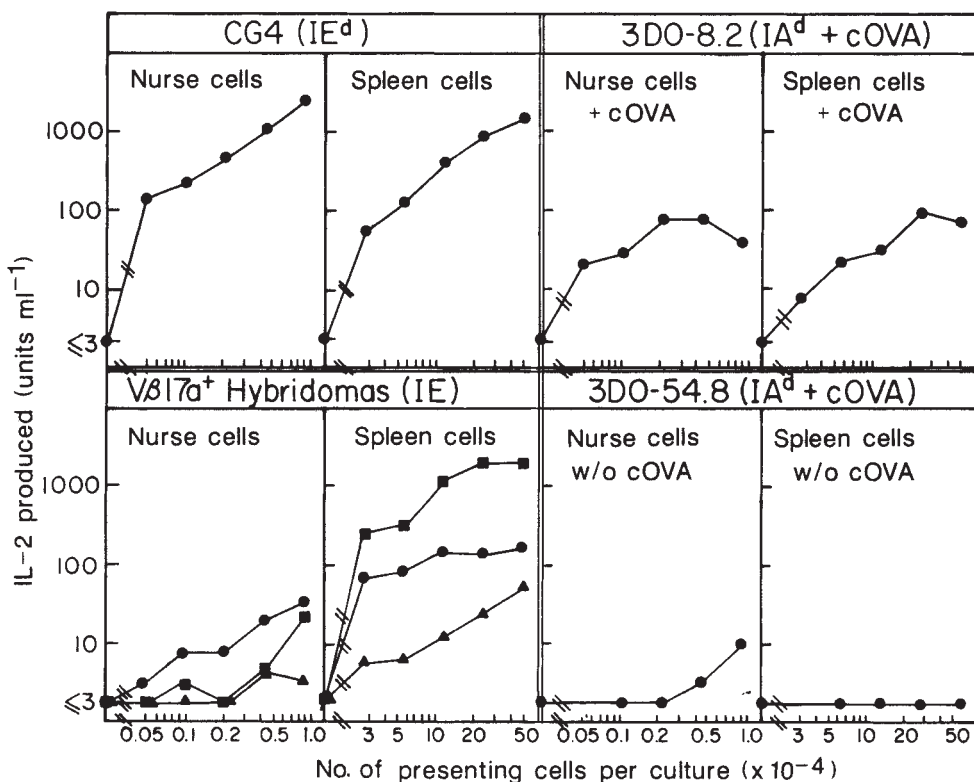
mal T-cell parent is absent, did not respond to syngeneic nurse cells, nor did a subclone of 3DO-54.8, 3DO-54.8.43, which no longer expresses CD4. It seems therefore that reaction of 3DO-54.8 with BALB/c nurse cells involves I-A^d on the nurse cells, CD4 and, probably, the BALB/c T-cell-derived receptor on 3DO-54.8.

Overall, these results indicate that MHC on thymus cortical epithelial cells is not always seen by T cells in the same way as MHC expressed on other cell types. Although nurse cells present antigen plus self-MHC, and in some cases allogeneic MHC, very effectively to some T cells, they are defective in their ability to present alloantigen to other cells: in the present case, I-E to V β 17a⁺ T cells. As the ligand for V β 17⁺ receptors is thought to be a complex of I-E and a B-cell-derived ligand¹⁵, it is not surprising that thymus cortical epithelial cells, which probably have limited access to B-cell products, do not present it effectively.

More interestingly, MHC on thymus cortical epithelial cells, but not on other cells, can in some cases stimulate hybridomas made from syngeneic T cells, a reaction which may reflect the process of positive selection. These syngeneic responses do not occur with all T cells and are very small, suggesting that the combination of positive selection and tolerance allows only those cells with very weak reactivity for self-MHC to develop into mature T cells, a possibility suggested previously by the work of Ron *et al.*^{10,11}. Alternatively, the weak, variable results may reflect the fact that our tests *in vitro* are poor models of the situation *in vivo*, where positive selection probably involves very intimate contact between thymus epithelium and CD4⁺, CD8⁺ thymocytes¹⁸. In either case, the response of T cells to syngeneic thymus epithelial cells, but not to spleen cells, may

FIG. 1 Presentation of antigen, allogeneic MHC and self by thymus cortical epithelial cells.

METHODS. Thymuses were removed from 40 6-week-old BALB/c mice, and nurse cells prepared as previously described¹², using a combination of mechanical disruption, digestion with collagenase (0.1 U ml⁻¹, from *Achromobacter iophagus*, Boehringer), dispase (0.8 U ml⁻¹, from *Bacillus polymixa*, Boehringer) and bovine pancreas DNase I (0.05 mg ml⁻¹), and gravity sedimentation on fetal bovine serum step gradients in the presence of 50 mM EDTA. In the experiment shown, 2.4×10^5 nurse cells were obtained, contaminated with an equal number of free thymocytes. Spleen cells were treated with anti-T-cell serum and complement to remove T cells before use as antigen-presenting cells. Nurse or spleen cells were titrated into 250 μ l microcultures containing 10^5 T hybridoma cells and cOVA, where required, at 1 mg ml⁻¹. After 24 h, supernatants from these cultures were assayed for interleukin-2 (IL-2) content using HT-2 cells and MTT as an indicator of cell survival^{19,20}. This method allows <2 U ml⁻¹ of IL-2 to be measured accurately. Control cultures, set up with nurse or spleen cells alone, or with a T-cell hybridoma with no reactivity for BALB/c targets, contained no detectable IL-2. The results shown are those from a single experiment, representative of five similar experiments. Pairs of panels show IL-2 titres with the indicated T-cell hybridomas assayed on nurse or spleen cells. CG4 was produced from a fusion of C57BL/6 anti-BALB/c T cells to BW5147, and is specific for I-E^d. 3DO-8.2 was produced from a fusion of BALB/c (H-2^d), cOVA-primed T cells to BW5147. In this experiment 3DO-8.2 showed



no response to BALB/c nurse or spleen cells in the absence of cOVA. 36-19-21 (■), 2Q23-34.7.9 (●) and Q023-26.9 (△) were three V β 17a⁺ T-cell hybridomas, produced by fusion of SWR T cells to an α -derivative of BW147 (ref. 17). The 3DO-54.8 was produced from a fusion of cOVA-primed, BALB/c T cells to BW5147. The hybridoma is specific for cOVA/I-A^d. In this experiment it gave 119 U/ml⁻¹ IL-2 when challenged with cOVA and BALB/c spleen cells.

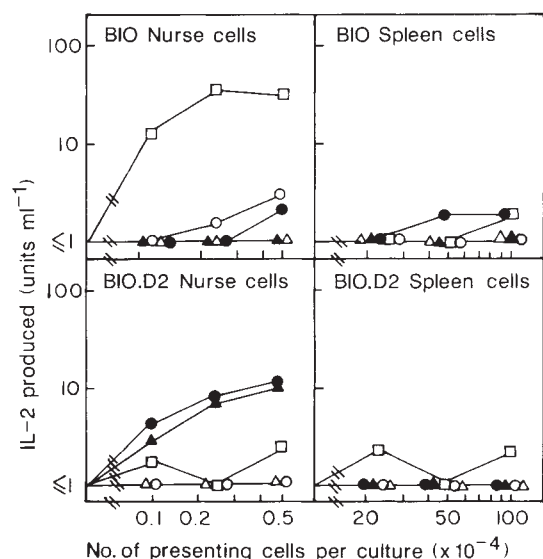


FIG. 2 Some T-cell hybridomas react specifically with syngeneic thymus cortical epithelial cells. Nurse cells were prepared from 19 B10 (H-2^b) or B10.D2 (H-2^d) 6–8-week-old mice as described in the legend to Fig. 1. These, and anti-T- and complement-treated spleen cells from the same animals, were tested for their ability to stimulate T-cell hybridomas bearing T-cell receptors from H-2^b (open symbols) or H-2^d (closed symbols) mice. Origins of the T-cell hybridomas: B0-97.10 (□) was produced from the fusion of cOVA-primed, C57BL/10 (B10, H-2^b) T cells to BW5147. 2B0-33 (Δ) and 2B0-43 (○) were also produced from cOVA-primed, B10 cells, but these were fused to an α⁻, β⁻-derivative of BW5147 (ref. 17). 3D0-54.8 (●) was produced as described in the Fig. 1 legend, 22D0-43 (▲) was produced by fusion of cOVA-primed B10.D2 (H-2^d) T cells to the α⁻, β⁻-derivative of BW5147.

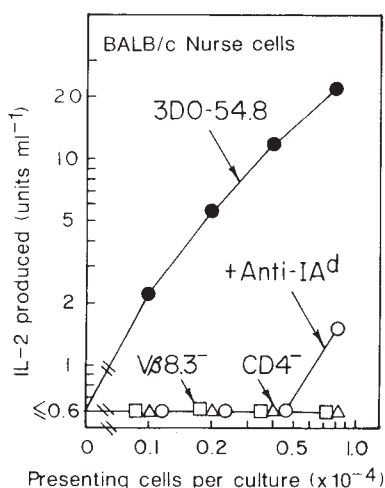


FIG. 3 The normal T-cell-derived receptor and CD4 are required for recognition of restricting I-A on syngeneic thymus cortical epithelial cells by a T-cell hybridoma.

METHODS. Nurse cells were prepared from 40 5-week-old BALB/c mice as described in Fig. 1 legend. These, and anti-T- and complement-treated spleen cells, were assayed for their ability to stimulate the BALB/c-derived T-cell hybridoma, 3D0-54.8 (●), and its derivatives, as described in Fig. 1. 3D0-54.8.21 (□) and 3D0-54.8.43 (Δ) were produced by subcloning after prolonged passage of 3D0-54.8 in culture. Southern blots showed that 3D0-54.8.21, which no longer reacts with cOVA/I-A^d, has lost the only normal T-cell-derived, rearranged β-chain gene characteristic of 3D0-54.8. 3D0-54.8.43, unlike its parent, bears no surface CD4, and reacts very weakly with cOVA/I-A^d. The anti-I-A^d monoclonal antibody, MK-D6 was used as a 20% concentration of culture supernatant from the antibody-producing cell line (○).

occur because of quantitative or qualitative differences in MHC expression between thymus epithelial and peripheral cells. We find that thymus cortical epithelial cells do not express a higher density of MHC proteins than cells such as some B-cell lymphomas. Of these two explanations, we therefore prefer the latter; but, of course, the two theories are not mutually exclusive, and both may apply for different T cells. □

Received 8 November 1988; accepted 22 February 1989.

1. Marrack, M. & Kappler, J. *Science* **238**, 1073–1079 (1987).
2. Babbitt, B., Matsueda, G., Haber, E., Unanue, E. & Allen, P. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4509–4513 (1986).
3. Buus, S., Sette, A., Colon, S., Miles, C. & Grey, H. *Science* **235**, 1353–1358 (1987).
4. Bjorkman, P. J. et al. *Nature* **329**, 506–512 (1987).
5. Bevan, M. & Fink, P. *Immunol. Res.* **42**, 3–19 (1978).
6. Yoshizaki, K. et al. *J. Immun.* **128**, 1296–1301 (1982).
7. Kisielow, P., Teh, H. S., Bluthmann, H. & von Boehmer, H. *Nature* **335**, 730–733 (1988).
8. Sha, W. C. et al. *Nature* **336**, 73–76 (1988).
9. Kappler, J., Roehm, N. & Marrack, P. *Cell* **49**, 273–280 (1987).
10. Lo, D., Ron, Y. & Sprent, J. *Immunol. Res.* **5**, 221–232 (1986).
11. Sprent, J. & Webb, S. *Adv. Immun.* **41**, 39 (1987).
12. Wekerle, H., Ketelsen, U.-P. & Ernst, M. *J. exp. Med.* **151**, 925–944 (1980).
13. Kyewski, B. A., Fathman, C. G. & Kaplan, H. S. *Nature* **308**, 196–199.
14. Kappler, J. et al. *Cell* **49**, 263–271 (1987).
15. Marrack, P. & Kappler, J. *Nature* **332**, 840–843 (1988).
16. Marrack, P. et al. *Cell* **53**, 627–634 (1988).
17. Born, W., White, J., O'Brien, R. & Kubo, R. *Immunol. Res.* **7**, 279–291 (1988).
18. Teh, H. S. et al. *Nature* **335**, 229–233 (1988).
19. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. *J. exp. Med.* **153**, 1198–1214 (1981).
20. Mosmann, T. *J. Immunol. Meth.* **65**, 55–63 (1983).

ACKNOWLEDGEMENTS. We thank Ella Kushnir, Rhonda Richards, Terri Wade and Janice White for their technical assistance, and Dr H. Grey for the gift of CG4. This work was supported by the USPHS.

Severe immunodeficiency disease induced by a defective murine leukaemia virus

Douglas C. Aziz^{*†}, Zaher Hanna^{*} & Paul Jolicoeur^{*‡§}

^{*} Laboratory of Molecular Biology, Clinical Research Institute of Montreal, 110 Avenue des Pins ouest, Montréal, Québec H2W 1R7, Canada

[‡] Département de Microbiologie et d'Immunologie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

DIFFERENT classes of retroviruses have been shown to induce immunodeficiency diseases in various animal species¹. These animal models may provide an insight into our understanding of AIDS^{1–3} but, with the exception of one strain of feline leukaemia virus⁴, the determinants of pathogenicity have not yet been mapped to these viral genomes. The immunodeficiency-inducing feline leukaemia virus is replication-defective⁴, harbouring the determinant of pathogenicity within its *env* sequences⁵. We have studied the Duplan strain of murine leukaemia virus⁶ which induces, in C57BL/6 mice^{7–11}, a severe immunodeficiency disease with striking similarities to human AIDS^{2,3,8,9}. We have identified the aetiological agent of this murine immunodeficiency disease as another defective retrovirus, with a genome of 4.8 kilobases. Molecular cloning and sequencing of this DNA showed that the *pol* and *env* genes have been deleted, but that the complete *gag* region has been conserved and has a novel sequence encoding the p12 protein. As with the feline leukaemia virus^{4,5}, these results provide evidence for the role of defective retroviruses in inducing immunodeficiency and facilitate the study of the mechanisms underlying the pathogenesis of retrovirus-induced immunodeficiency syndromes, including AIDS.

A crude extract containing a mixture of different strains of Duplan murine leukaemia viruses (MuLVs), prepared from a lymph node of a diseased mouse, was used to infect SC-1 cells *in vitro*. Filtered supernatant (SC-1/Dup MuLV) from the

[†] Present address: Cytometrics Inc., 11575 Sorrento Valley Road, San Diego, California 92121, USA.
[§] To whom correspondence should be addressed.

infected cells induced typical disease within 8 to 12 weeks of inoculation of young adult C57BL/6 mice (Table 1).

To analyse individual viruses present in this crude extract and to identify the retrovirus strain involved in the disease, we first analysed unintegrated viral DNA extracted by the Hirt procedure from SC-1 cells acutely infected with SC-1/Dup MuLV. Using a representative retroviral probe, we detected two

major species of linear viral DNA of 8.8 and 4.8 kilobases (kb), corresponding to a typical full-length non-defective MuLV genome, and to a defective retroviral genome, respectively (Fig. 1a, lane 1). This defective viral genome seemed to be as abundant as the non-defective species in this Hirt supernatant. The viral DNA species in the Hirt supernatant were separated into supercoiled and linear or open-circular viral DNA. The defective

TABLE 1 Biological characteristics of cloned Du5H defective MuLV

Virus injected	Mice with lymphadenopathy and splenomegaly	[³ H]thymidine incorporation (c.p.m. $\times 10^{-3}$)				Serum IgM (mg ml ⁻¹)
		0	2.5	5.0	10.0	
No virus	0 (8)	2.8 $\pm$ 0.4	84 $\pm$ 17	148 $\pm$ 18	118 $\pm$ 22	0.25 $\pm$ 0.03
G ₆ T ₂	0 (8)	6.4 $\pm$ 3.0	72 $\pm$ 35	152 $\pm$ 36	152 $\pm$ 26	0.30 $\pm$ 0.01
Du5H (G ₆ T ₂)	8 (8)	1.4 $\pm$ 0.2	1.4 $\pm$ 0.3	1.6 $\pm$ 0.3	2.0 $\pm$ 0.5	1.60 $\pm$ 0.40
Duplan extract	8 (8)	1.8 $\pm$ 0.5	2.0 $\pm$ 0.5	2.1 $\pm$ 0.5	2.3 $\pm$ 0.4	1.65 $\pm$ 0.15

pDu5HNeo DNA was transfected into SM.R cells by the calcium phosphate method¹⁸ and neomycin-resistant colonies were grown in the presence of Geneticin (G418) (200 μ g ml⁻¹). Twenty-two colonies were picked and rescued with non-pathogenic (G₆T₂ RadLV (ref. 12). To select for the best producer, viruses were collected from the supernatant of each clone, recovered by centrifugation (50 Ti, 35,000 r.p.m. for 30 min) and their RNA extracted by the urea-SDS method¹⁹. Viral RNA was hybridized with the ³²P-labelled Du5H-specific D30 probe. The best producer was selected to produce stocks. As estimated by hybridization, these stocks contained an approximately three-fold higher concentration of helper viruses than Du5H viruses and the syncytium XC titre²⁰ of the helper MuLV was 5×10^3 plaque-forming units per ml. Young adult (30–40-day-old) C57BL/6 mice were injected intraperitoneally with 0.5 ml twice, one week apart, with medium alone, G₆T₂ RadLV alone, Du5H rescued with G₆T₂ RadLV, or with the crude extract of Duplan RadLV. Mice were killed 12–17 weeks after injection when they showed advanced signs of disease (lymphadenopathy). Peripheral lymph-node size varied from 0.4 to 1.5 cm. Spleen cells were suspended into RPMI 1640 medium (Gibco) with 10% fetal calf serum, penicillin G (Sigma) 100 units ml⁻¹, streptomycin (Sigma) 100 mg ml⁻¹ and 10^{-5} M β -mercaptoethanol. Aliquots containing 2×10^5 cells per 200 μ l were distributed in wells of microtest plates (Costar) and incubated at 37 °C for 48 h in humidified atmosphere containing 5% CO₂ with concanavalin A (Con A, Sigma) at 2.5, 5.0 or 10 μ g ml⁻¹. Cells were incubated for 4 h with 1 μ Ci of [³H]thymidine (42 Ci mmol⁻¹) (Amersham) and collected in an automated sample harvester (PHD, Cambridge Technology). Incorporated precursor was counted in a scintillation spectrometer (1215 Rack Beta II, Wallac). The standard error was calculated from an average of 3–5 mice. The concentration of IgM in the sera was measured by a competitive enzyme-linked immunosorbent assay. Polystyrene microtitre plates (Nunc) were coated with affinity-purified mouse IgM (Sigma). Serial dilutions of serum from each mouse were assayed for its ability to inhibit the binding of alkaline phosphatase-conjugated, affinity-purified rabbit anti-mouse IgM (ICN). *p*-nitrophenyl disodium phosphate (Sigma; 1 mg ml⁻¹) was used as a substrate in IM diethanolamine-HCl (pH 9.8) and 50 mM MgCl₂ and the colour read in the Biotek Model 310 Autoreader at 405 nm. Standard inhibition curves were compared with competitive binding with unconjugated mouse IgM. The standard error was calculated from an average of 3–5 mice.

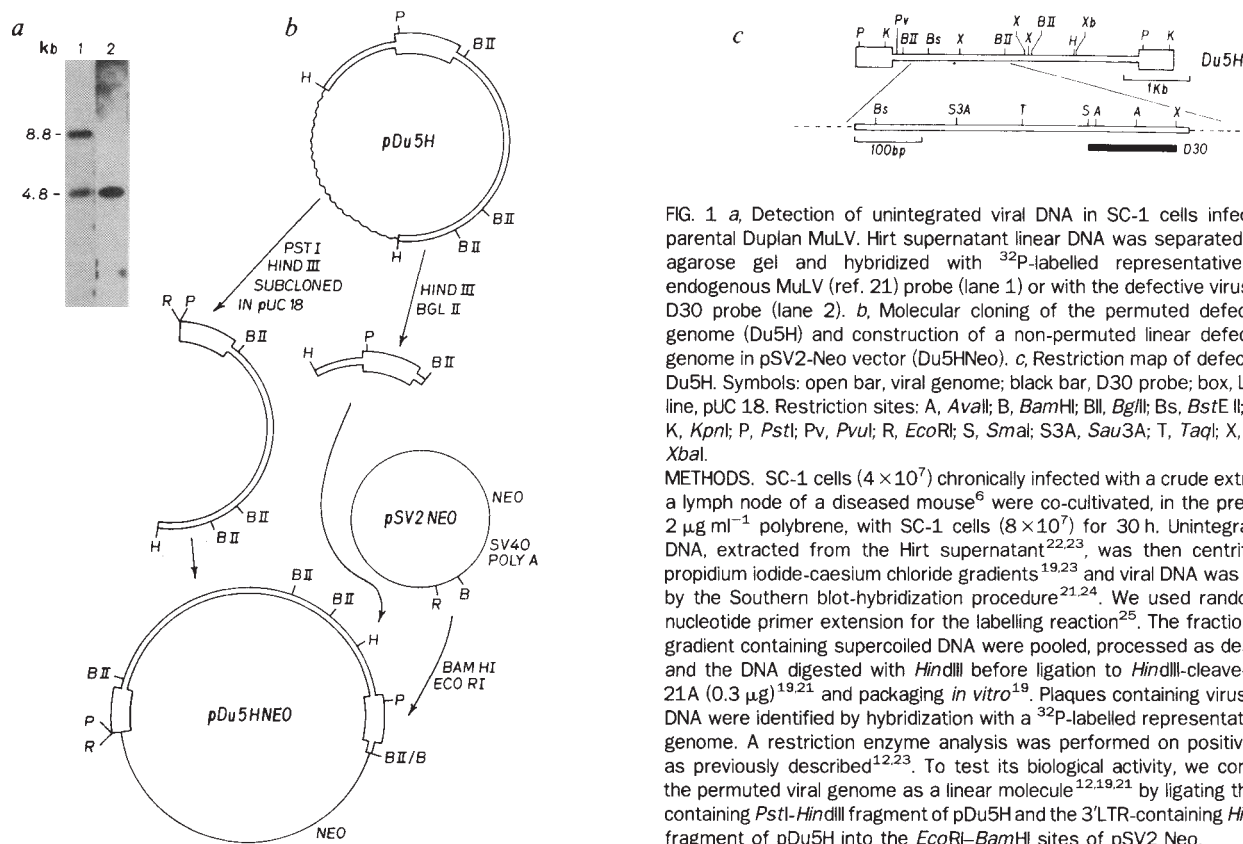
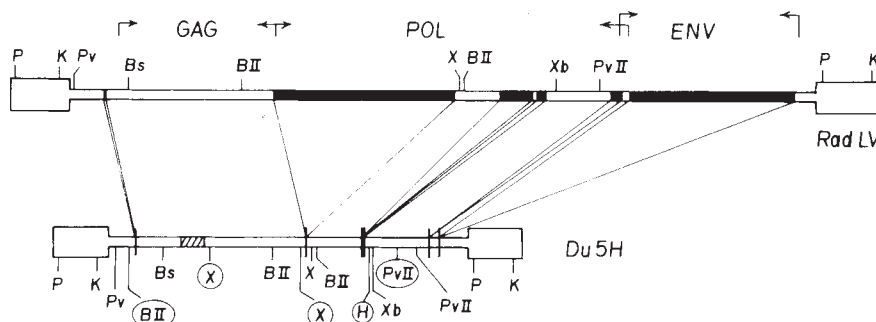


FIG. 1 a, Detection of unintegrated viral DNA in SC-1 cells infected with parental Duplan MuLV. Hirt supernatant linear DNA was separated on 0.7% agarose gel and hybridized with ³²P-labelled representative BALB/c endogenous MuLV (ref. 21) probe (lane 1) or with the defective virus-specific D30 probe (lane 2). b, Molecular cloning of the permuted defective viral genome (Du5H) and construction of a non-permuted linear defective viral genome in pSV2-Neo vector (Du5HNeo). c, Restriction map of defective virus Du5H. Symbols: open bar, viral genome; black bar, D30 probe; box, LTR; wavy line, pUC 18. Restriction sites: A, *Ava*I; B, *Bam*HI; BII, *Bgl*II; Bs, *Bst*E II; H, *Hind*III; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*I; R, *Eco*RI; S, *Sma*I; S3A, *Sau*3A; T, *Taq*I; X, *Xho*I; Xb, *Xba*I.

METHODS. SC-1 cells (4×10^7) chronically infected with a crude extract from a lymph node of a diseased mouse⁶ were co-cultivated, in the presence of 2 μ g ml⁻¹ polybrene, with SC-1 cells (8×10^7) for 30 h. Unintegrated viral DNA, extracted from the Hirt supernatant^{22,23}, was then centrifuged on propidium iodide-caesium chloride gradients^{19,23} and viral DNA was detected by the Southern blot-hybridization procedure^{21,24}. We used random hexanucleotide primer extension for the labelling reaction²⁵. The fractions of the gradient containing supercoiled DNA were pooled, processed as described²³ and the DNA digested with *Hind*III before ligation to *Hind*III-cleaved Charon 21A (0.3 μ g)^{19,21} and packaging *in vitro*¹⁹. Plaques containing virus-specific DNA were identified by hybridization with a ³²P-labelled representative MuLV genome. A restriction enzyme analysis was performed on positive clones, as previously described^{12,23}. To test its biological activity, we constructed the permuted viral genome as a linear molecule^{12,19,21} by ligating the 5'LTR-containing *Pst*I-*Hind*III fragment of pDu5H and the 3'LTR-containing *Hind*III-*Bgl*II fragment of pDu5H into the *Eco*RI-*Bam*HI sites of pSV2 Neo.

FIG. 3 Comparison of the restriction map of the defective Du5H viral genome with that of non-defective RadLV (refs 12, 13). Common restriction enzyme sites are indicated. Several large deletions are present in *pol* and *env*. The *gag* sequences are homologous in p15, p30 and p10. Symbols: white box, homologous; black box, deleted; hatched box, unique sequences. BII, *Bgl*II; Bs, *Bst*E II; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*I; PvII, *Pvu*II; X, *Xho*I; Xb, *Xba*I; Circled restriction endonuclease sites are sites present in Du5H, but not in RadLV.



(G₆T₂) MuLV, but not in mice injected with helper (G₆T₂) alone (Table 1). Serum IgM was also markedly elevated in the Du5H (G₆T₂) MuLV-inoculated diseased mice, as compared with the controls (Table 1). Therefore, the defective Du5H genome pseudotyped with a non-pathogenic helper MuLV was sufficient to induce the same disease as the crude Duplan extract, namely lymphadenopathy, splenomegaly, immune suppression and hypergammaglobulinaemia.

To study the structure of this defective viral genome in more detail, we obtained its nucleotide sequence (Fig. 2). Comparison of this sequence with that of other RadLVs (refs 12, 13) revealed several large deletions in *pol* and *env* and a relatively well conserved region in *gag* (Fig. 3). The LTR sequences are very similar to those of the endogenous ecotropic MuLV from C57BL/6 mouse¹², diverging by only six point mutations in the region U3, one in R and three in the U5 region. They contain only one 99-bp direct repeat in U3. The sequences corresponding to *gag* p12 and to the D30 probe diverged the most from those of other MuLVs. Four large putative open reading frames could be recognized. Three (175, 173 and 170 amino acids; nucleotides 1,175–1,699, 3,241–3,759 and 3,469–3,979, respectively), correspond to part of *gag*, part of *pol* and part of *pol-env*, respectively. The fourth and largest open reading frame (nucleotides 802–2,559) has an ATG codon corresponding to the N-terminus of Gag p15 (nucleotide 970) and continues to p15, p12, p30 and p10. The p15, p30 and p10 showed respectively 81%, 95% and 95% identity to the corresponding amino-acid sequence of AKR MuLV (AKV) (ref. 14), the sequence in the data banks that was closest to it. The Du5H p12 is different from VL3-RadLV (ref. 13) or AKV (ref. 14) p12, however, being shorter by eight amino-acid residues and diverging most notably in its 50-amino acid C-terminus. This unique amino-acid sequence is rich in prolines and has many basic amino-acid residues. A computer search of current entries in sequence libraries revealed that the Du5H Gag p12 C-terminal region had only 40–50% identity with Gag p12 protein of other murine retroviruses, and was not identical to any other non-retroviral sequences in the database.

The pathogenic virus present in the crude Duplan RadLV extract is a unique defective retrovirus capable of inducing a severe immunodeficiency disease after rescue with a non-pathogenic helper ecotropic RadLV. This virus represents the second example, in addition to the variant defective FeLV^{4,5}, of an immunodeficiency-inducing retrovirus that is replication-defective. Our results re-emphasize⁵ the need to search for similar pathogenic replication-defective variants in other immunodeficiency diseases, including AIDS, and suggest that the pathogenic AIDS virus may also be a defective retrovirus.

The Duplan defective RadLV genome has deleted its *env* region and has a well conserved *gag* region with a unique p12 sequence encoding a putative novel protein. Because this p12-related protein is unique and because of the absence of any other unique long open reading frame, this *gag* region is probably important in the pathogenesis of the disease. Only one

retrovirus, the avian osteopetrosis virus, is known to contain its chief determinant of pathogenicity within its *gag* region¹⁵. The origin of the unique and shorter p12-related sequence of Duplan defective RadLV remains unclear. It may be derived by recombination between genomic sequences and a primary non-defective RadLV¹², or it might have evolved by multiple modifications (point mutations, deletions, substitutions and so on of a known *gag* p12 sequence).

The mechanism by which this defective virus induces immunodeficiency remains to be determined, but the similarities of this syndrome with human AIDS are striking. Both viruses induce polyclonal B-cell proliferation, manifested by lymphadenopathy, splenomegaly and hypergammaglobulinaemia, as well as severe immunodeficiency (involving both T-lymphocyte and B-lymphocyte functions), enhanced susceptibility to infections and terminal B-cell lymphomas^{2,3,6–11,16}. The presence of T lymphocytes was found to be essential to the development of the murine disease¹⁷. Therefore, a common perturbation or perturbation of a common pathway may underline this murine syndrome and human AIDS. Further study of this system may lead to better understanding of the pathogenesis of retrovirus-induced immunodeficiency diseases, including AIDS. □

Received 24 October 1988; accepted 17 February 1989.

- Desrosiers, R. C. & Letvin, N. L. *Rev. Infect. Dis.* **9**, 438–446 (1987).
- Wong-Staal, F. & Gallo, R. C. *Nature* **317**, 395–403 (1985).
- Fauci, A. S. *Science* **239**, 617–622 (1988).
- Mullins, J. I., Chen, C. S. & Hoover, E. A. *Nature* **319**, 333–336 (1986).
- Overbaugh, J., Donahue, P. R., Quackenbush, S. L., Hoover, E. A. & Mullins, J. I. *Science* **239**, 906–910 (1988).
- Latarget, R. & Duplan, J. F. *Int. J. Radiat. Biol.* **5**, 339–344 (1962).
- Legrand, E., Daculsi, R. & Duplan, J. F. *Leukemia Res.* **5**, 223–233 (1981).
- Mosier, D. E., Yetter, R. A. & Morse III, H. C. *J. exp. Med.* **161**, 766–784 (1985).
- Mosier, D. E. *Immun. Invest.* **15**, 233–261 (1986).
- Klinken, S. P., Fredrickson, T. N., Hartley, J. W., Yetter, R. A. & Morse III, H. C. *J. Immun.* **140**, 1123–1131 (1988).
- Buller, R. M. L., Yetter, R. A., Fredrickson, T. N. & Morse III, H. C. *J. Virol.* **61**, 383–387 (1987).
- Rassart, E., Shang, M., Boie, Y. & Jolicoeur, P. *J. Virol.* **58**, 96–106 (1986).
- Merregaert, J., Janowski, M. & Reddy, E. P. *Virology* **158**, 88–102 (1987).
- Herr, W. *J. Virol.* **49**, 471–478 (1984).
- Robinson, H. L., Reinsch, S. S. & Shank, P. R. *J. Virol.* **59**, 45–49 (1985).
- Pattengale, P. K. *et al. Am. J. Pathol.* **107**, 362–377 (1982).
- Mosier, D. E., Yetter, R. A. & Morse III, H. C. *J. exp. Med.* **165**, 1737–1742 (1987).
- Graham, F. L. & van der Erb, A. J. *Virology* **52**, 456–467 (1973).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Rowe, W. P., Pugh, W. E. & Hartley, J. W. *Virology* **42**, 1136–1139 (1970).
- Rassart, E., DesGroselliers, L. & Jolicoeur, P. *J. Virol.* **39**, 162–171 (1981).
- Hirt, B. *J. molec. Biol.* **26**, 365–369 (1967).
- Jolicoeur, P. & Rassart, E. *J. Virol.* **33**, 183–195 (1980).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Feinberg, A. P. & Volgelstein, B. *Analyt. Biochem.* **132**, 6–13 (1983).
- Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. S. H. & Roe, B. A. *J. molec. Biol.* **143**, 161–178 (1980).
- Chen, E. Y. & Seeburg, P. H. *DNA* **4**, 165–170 (1985).

ACKNOWLEDGEMENTS. Sequence data from this manuscript will appear in the EMBL/GenBank/DBJ Nucleotide Databases under accession number X14576 DU 5H. This work was supported by grants to P. J. from the Medical Research Council of Canada and from the National Cancer Institute of Canada. D. C. A. was a recipient of a Fellowship from the National Cancer Institute of Canada. We thank Ginette Massé and Benoit Laganière for technical assistance, Izabella Gorska for help in sequencing DNA, and Drs Jean-François Duplan and Bernard Guillemain for providing the original virus extract.

Alternative splicing of human dystrophin mRNA generates isoforms at the carboxy terminus

Chris Anne Feener*, Michel Koenig*†
& Louis M. Kunkel*†

* Division of Genetics and Mental Retardation Center, and † Howard Hughes Medical Institute: The Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA

DYSTROPHIN is the protein product of the Duchenne/Becker muscular dystrophy locus. It has a relative molecular mass of 427,000 and is encoded by a large RNA transcript processed from more than 65 exons spread over two million base pairs of the human X chromosome¹⁻⁵. We have used the polymerase chain reaction⁶ to see whether any of these exons are used alternatively in the different tissues that express dystrophin. As reported for rat dystrophin⁷, we find that the first exon of the human dystrophin transcript is different in brain and muscle, indicating that dystrophin expression could be differentially regulated in these tissues by usage of distinct promoters. The 3' end of the dystrophin transcript can be alternatively spliced to create numerous isoforms differing at their carboxyl domains; this is the only domain of dystrophin that does not share any similarity with the related cytoskeletal α -actinins³. These alternative transcripts yield dystrophin molecules which may interact with different proteins of the tissues expressing dystrophin.

Northern blot analysis of dystrophin messenger RNA from different tissues did not reveal heterogeneity of the transcript in any given tissue, or from one tissue to another (refs 8, 9 and unpublished observations), although minor size variations are difficult to detect. Western blots reveal a predominant protein band corresponding to M_r 427K for dystrophin¹. Smaller forms are detectable however, and these could represent immunocross-reactive proteins, partial proteolytic breakdown products or isoforms of dystrophin^{1,10}. One of these smaller forms (M_r ~405K) of dystrophin is predominant in smooth muscle of mouse¹⁰. To test whether alternative splicing is responsible for these differences in protein size, we used the polymerase chain reaction (PCR) (ref. 6) to screen different regions of the human dystrophin mRNA for lost or gained exons. Twelve pairs of primers, derived from the dystrophin complementary DNA sequence³, were designed to amplify overlapping segments of ~1 kilobase spanning the entire dystrophin coding sequence. Amplified fragments were visualized directly on polyacrylamide gels or on agarose gels which were Southern blotted and hybridized with cloned skeletal muscle dystrophin cDNA. Any amplified segment of a size not predicted from sequence analysis would indicate a variation resulting from alternative splicing. All segments were amplified from single-stranded cDNA prepared from four different human tissues known to express dystrophin: skeletal muscle (psoas or leg muscles), cardiac muscle, smooth muscle (stomach or aorta) and brain. Tissues expressing lower levels of dystrophin¹¹ were not analysed.

The sizes of the amplified fragments corresponding to segments 2 to 10 were as predicted from the sequence of dystrophin cDNA from skeletal muscle³ and were identical for all four tissues tested (Fig. 1*b* and *c*). The predicted fragments for the other segments were not uniquely observed in each tissue. The amplified fragment corresponding to segment 1 was found in skeletal, cardiac and smooth muscle, but not in brain tissue (Fig. 1*a*). This result indicated that the sequence of one of the two primers used to amplify this 5' segment could be absent in the major form of the brain dystrophin transcript. Subsequent blotting of the gel and hybridization with a dystrophin cDNA probe revealed traces of the expected fragment in the brain reaction, possibly as a result of the smooth muscle content of brain (data

not shown). The fragment expected for segment 11 was found in all the tissues we tested, but a smaller and less abundant fragment was also co-amplified (Fig. 1*d*). The pattern of fragments obtained for segment 12 was more complex. To map this rearranged region more precisely, we amplified segment 12 as two smaller overlapping sub-segments. The amplification of the 5' sub-segment, 12a (Fig. 1*e*), gave the expected fragment and three other fragments that were smaller by ~40, 100 and 330 base pairs (bp). The amplification of the 3' sub-segment, 12b

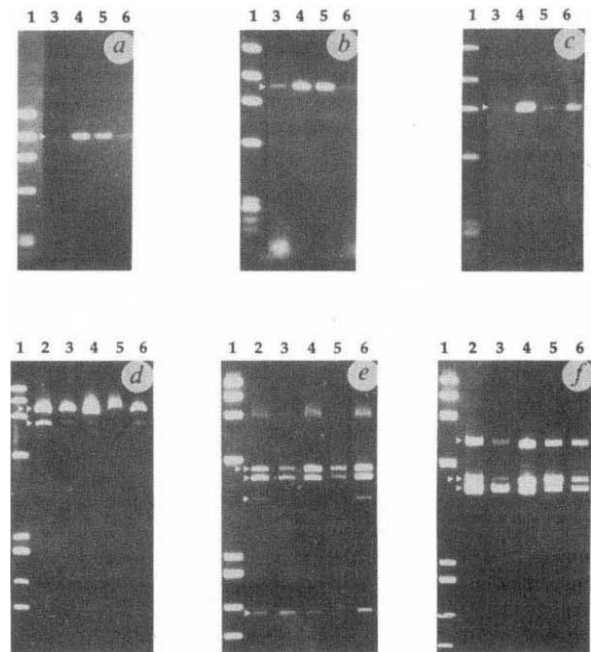


FIG. 1 PCR amplification of six segments of the dystrophin transcript. Amplified products were separated on 1.2% agarose gels (*a-c*) or on 4% polyacrylamide gels (*d-f*). Lane 1 is Φ X174 replicative form DNA/*Hae*III-cut standard (BRL); lanes 2, 3, 4, 5 and 6 are amplification products of human fetal aorta, brain, heart, leg and stomach tissue respectively. *a*, Amplification product of segment 1 (position relative to the published cDNA sequence 141-1,207); *b*, segment 5 (3,770-4,771); *c*, segment 8 (6,602-7,462); *d*, segment 11 (9,395-10,342); *e*, segment 12a (10,288-10,840); *f*, segment 12b (10,811-11,350). The fragments expected from the skeletal muscle cDNA sequence are indicated by a single white arrow, or a double white arrow when more than one fragment is apparent. The expected fragment of segments 1, 5 and 8 (1,067, 1,002 and 861 bp respectively) is observed in all tissues, except for segment 1 in brain (Fig. 1*a*, lane 3). In *d*, a 948-bp fragment is detected along with an unexpected fragment of ~800 bp in length. The lower band appears to be more prevalent in aorta (lane 2) than in other tissues. In *e*, the expected amplified fragment of 553 bp is seen from all tissues, along with three other fragments of ~510, 450 and 225 bp respectively (material in lane 5 was obtained from a separate experiment). The 553-bp fragment is more prevalent in leg and cardiac muscle, whereas the most prevalent fragment from smooth muscle and brain is 510-bp. The 450- and 225-bp fragments also appear more in smooth muscle and brain than in striated muscles. In segment 12b (*f*) there are three amplified products (700, 540 and 510 bp). The more prevalent fragment in smooth muscle and brain samples is 510 bp, whereas the expected 540-bp fragment is more prevalent in cardiac and skeletal muscle. We are analysing the 700-bp fragment at present: it could represent use of a new 160-bp exon or simply be an artefact of the PCR, which is supported by the fact that it is not seen under different electrophoresis conditions.

METHODS. Total mRNA was isolated by guanidine isothiocyanate extraction¹² and was reverse-transcribed into single-strand cDNA using oligo (dT) (18-25) as primer and reverse transcriptase from Moloney murine leukaemia virus (BRL). Samples were extracted with phenol/chloroform and ethanol-precipitated. The PCRs used 30-mer oligonucleotides (Biopolymers Laboratory, Howard Hughes Medical Institute at Harvard Medical School) which had been purified by electrophoresis on a 20% denaturing polyacrylamide gel. PCR conditions were as recommended (Cetus Corporation), using ~40 ng cDNA and 1 μ g of each primer. Annealing and primer extension were at 72°C, to minimize artefactual priming, and the denaturation step was at 94°C.

(Fig. 1f), gave three fragments, one of the predicted size, one ~35 bp smaller, and another ~160 bp larger. These results indicate that there are three distinct regions involved in potential alternative splicing in the last 2 kb of the coding sequence. The relative proportions of each fragment within a single sample in segments 11, 12a and 12b differed from tissue to tissue. Analysis of the relative abundance of the fragments indicated that the fragments predicted from the 'skeletal muscle' sequence were indeed more abundant in striated muscles (skeletal and cardiac), whereas the other fragments were usually more abundant in smooth muscle and/or brain.

To investigate the absence of amplification product for segment 1 in brain, we isolated three brain cDNA clones using a 5' skeletal muscle cDNA probe. The sequence of one clone matched the skeletal muscle sequence over the second and third exon, but differed over the entire length of the first exon (Fig. 2a), whereas the sequence of two clones matched the sequence of skeletal muscle cDNA³ perfectly. The sequence of the alternative first exon indicates that this exon has an in-frame stop codon, followed further downstream by an initiation codon in-frame with the rest of the coding sequence. The alternative first exon would therefore encode only 2½ amino acids (Fig. 2a), replacing the 10½ amino acids of the muscle first exon. To confirm that an alternative first exon is used in brain tissue, we used a primer specific for the alternative exon for the PCR amplification of the corresponding transcript, along with parallel amplification of the 5' end of the skeletal muscle transcript (Fig. 3a and b). We found the alternative first exon in the main form of the brain transcript but also in transcripts of skeletal, cardiac and smooth muscles (Fig. 3b). This same exon has been found in rat brain transcripts⁷. It is striking that the 5' (apparently) untranslated

portion of this exon is highly conserved in human and rat (99% homology; Fig. 2a). In comparison, the 'skeletal muscle' first exon is only 80% homologous between human and mouse². A different first exon in brain implies that there are alternative promoter and regulatory sequences in brain and muscle, which could put expression of dystrophin in these tissues under different control systems.

We investigated the multiple fragments obtained by amplification of segments 11, 12a and 12b by direct sequencing with nested primers. In addition, the sequence of several cDNA clones isolated from skeletal muscle and brain cDNA libraries was determined over the regions involved in differential splicing. In all cases, fragments smaller than those predicted for 'skeletal muscle' resulted from loss of one or more exons, with no replacement of new nucleotide sequences (Fig. 4). The loss of exons in segment 12a maintained the reading frame but the loss of exons in segments 11 and 12b modified the reading frame, resulting in potential coding for new amino-acid sequences. Interestingly, the exon loss in segment 11 results in deletion of the entire carboxyl domain of dystrophin, and the 5' border of the deletion corresponds exactly to the end of the sequence similarity of dystrophin with the carboxyl domain of α -actinin³. Such dystrophin molecules would be formed only by the three domains that are evolutionarily conserved with α -actinin and might represent an ancestral form of the molecule.

The sequence of skeletal muscle and brain cDNAs confirmed the existence of the most abundant forms of transcript in the two tissues (Fig. 4). However, one skeletal muscle clone has been described as containing a portion of a putative unspliced intron³ (the position of the splice site was misindicated in Fig. 1 of this ref.) and one of the brain cDNA clones has the same

FIG. 2 5'-Terminal sequence of a brain cDNA clone, including the 'brain' first exon and putative intron sequence found in muscle and brain cDNAs. Exon boundaries are indicated by black triangles and relevant stop codons are underlined. a, Sequence of the first, second and part of third exon of human brain cDNA. The equivalent sequence from rat⁷ is shown for comparison. Mismatches are boxed. An ATG codon in frame with the coding sequence is found 7 nucleotides upstream from the first exon border and is preceded by a stop codon in the same reading frame. b, Sequence of the 3' end of two independent cDNA clones containing the same putative intron. The position where the sequence diverges from the skeletal muscle sequence is immediately followed by a typical donor splice site (boxed). The position of divergence is also the 5' border of the coding sequence deletions observed in segment 12a (see text). Numbering is according to the skeletal muscle cDNA sequence³. cDNA library construction and screening have been described^{2,8}. Both strands of the inserts subcloned into pBR322 or bluescript vectors were sequenced with the Sequenase kit (USB) using universal primers and cDNA-specific oligonucleotide primers.

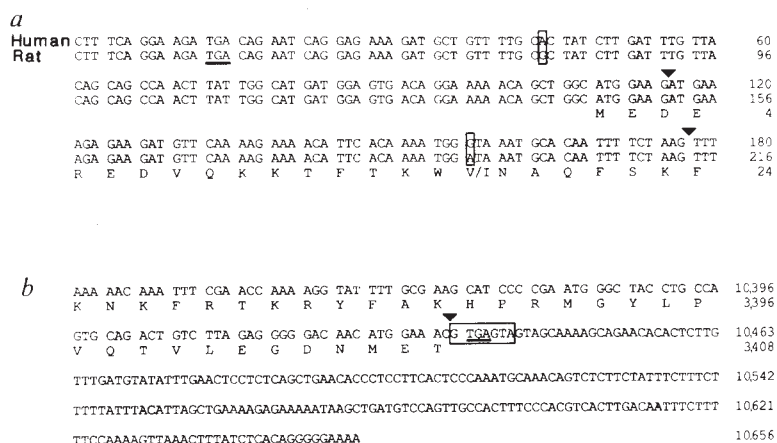


FIG. 3 Dystrophin transcript amplification using primers from the alternative first exon and from the putative 3' intron. Amplified fragments were separated on polyacrylamide gels. Lane 1 shows ϕ X174 replicative form DNA/HaeIII-cut standard size markers, as in Fig. 1. Lanes 2, 3, 4, 5 and 6 contain cDNA from human fetal aorta, brain, cardiac, leg muscle and stomach respectively. a and b, Amplification with a 5' primer from the skeletal muscle first exon gives no detectable fragment in the brain reaction (lane 3, panel a), whereas amplification with a 5' primer from the alternative first exon produced the expected 750-bp fragment (white arrow) not only in the brain reaction (lane 3, panel b) but also in the cardiac and leg muscle reactions (lanes 4 and 5, panel b). c, Coamplification of segment 12a with two different 3' primers. One 3' primer is from the dystrophin coding sequence (as in Fig. 1) and the other 3' primer is from the putative intron sequence (positions 10,570 to 10,599 in Fig. 2b). Amplification with the intron 3' primer produced the expected 312-bp fragment (dark arrow) in addition to the four fragments obtained with the coding sequence primers (white arrows; compare with Fig. 1e). RNA preparation, cDNA synthesis and PCR amplification are described in the legend to Fig. 1.

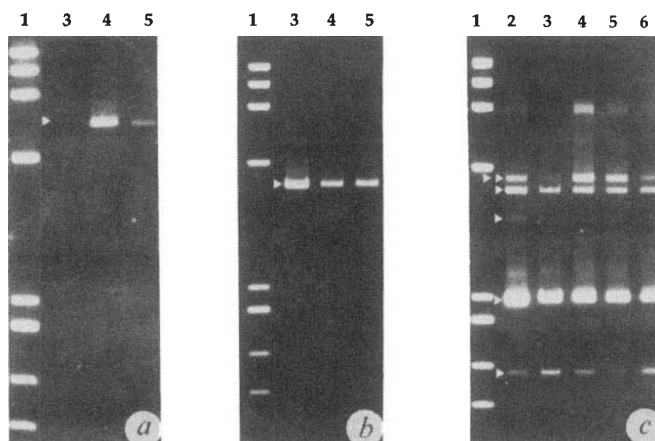
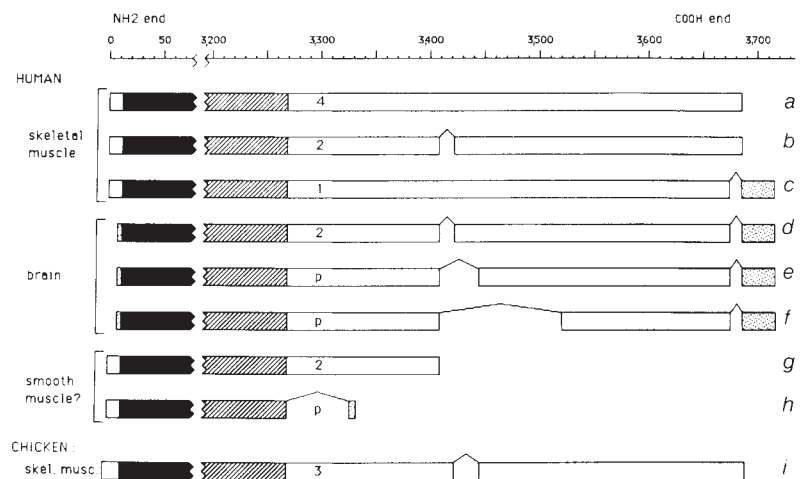


FIG. 4 Summary of possible protein forms encoded by the transcript variants described in this study. N- and C-termini of the dystrophin proteins are represented by bars labelled *a* to *i*. The black and hatched bars represent portions of the domains homologous to the amino and carboxyl domains of dictyostellium α -actinin respectively. Dotted bars represent amino-acid sequences differing from the published dystrophin sequence. The large open bar represents the carboxyl domain of dystrophin. The numbers indicated in these open bars are the numbers of cDNA clones isolated from brain and skeletal muscle cDNA libraries encoding the corresponding form of the carboxyl domain. *p*, Forms that have been identified solely from the sequence of fragments amplified by PCR. The different forms are not strictly tissue-specific and other forms corresponding to combinations of differentially spliced exons are possible. The tissues in which a particular form is thought to be predominant are shown on the left; 'skeletal' refers to both skeletal and cardiac muscles. The scale is numbered by amino-acid residues and is shown at the top of the diagram. The description of the skeletal muscle form of the chicken dystrophin (*i*) is from Lemaire *et al.*¹⁴. The different rearrangements at the origin of the forms (*a* to *i*) is as follows: *a*, human skeletal muscle consensus form; *b*, 39-bp deletion (positions 10,432 to 10,470); *c*, 32-bp deletion (11,223 to 11,254); *d*, 'brain' first exon and 39- and 32-bp deletions; *e*, 'brain' first exon, 32-bp deletion and 105-bp deletion (10,432 to 10,536); *f*, 'brain' first exon, 32-bp deletion and 330-bp deletion (10,432 to 10,761); *g*, putative intron sequence (starting at position 10,432, see Fig. 3); *h*, 167-bp deletion (10,016 to 10,182); *i*, 66-bp deletion (positions 10,471 to 10,536 relative to the human cDNA map).

METHODS. Cloning and sequencing of the cDNA clones is described in the legend to Fig. 2. Fragments from PCR amplification of segments 11, 12a and 12b were gel-purified before sequence determination. As the different fragments were present in all tissues, they were prepared from the tissue where they were most abundant. The fragments detected in segments 12a and 12b were purified from reactions using brain and heart material and



fragments from segment 11 from aorta. Typically, 5 PCR reactions were pooled and ethanol-precipitated with 0.1% SDS and tRNA as carrier and electrophoresed on 1% low-melting point agarose gels or 4% polyacrylamide gels. Fragments were cut from the gels and either directly purified and concentrated with the GeneClean kit (Bio101) (agarose gels) or electro-eluted in dialysis bags and then purified and concentrated with the GeneClean kit (polyacrylamide gels). After boiling, fragments (100 ng) were directly sequenced with the Sequenase kit ('dGTP' mixes, USB) and S35-dATP (>1,000 Ci mmol⁻¹, Amersham). In some cases the concentration of the respective deoxynucleotides in the termination mixes was increased from 8 to 32 mM to maximize the elongation of molecules over the size range of interest (between 100 and 200 nucleotides). The entire sequencing reaction was run on a 6% polyacrylamide gel and exposed for 7 days.

intron sequence (Fig. 2*b*). Interestingly, the splice site starts at the same position as the deletion variations of segment 12a. Two explanations would account for the finding of two independent clones, apparently representing the same intermediate form of splicing. The splicing machinery could pause at this key intron, resulting in a substantial accumulation of the intermediate form. Alternatively, the intron could be part of a mature transcript. The coding sequence of this transcript terminates at a TGA stop codon contained in the putative donor splice site (see Fig. 2*b*). The transcript could therefore encode a dystrophin with the last 277 amino acids missing (7.5% of the entire protein), a size corresponding to the smaller form of dystrophin seen on western blots¹⁰. To confirm the natural occurrence of the putative unspliced intron found in the two cDNA clones, we made a primer from the intron sequence and used it with the previous primers to amplify segment 12a. We detected the predicted 312-bp fragment representing this unusual transcript in all tissues (most prominent in the aorta sample), together with the four fragments seen previously (Fig. 3*c*). The presence of this 312-bp form in all tissues could be explained either by the smooth muscle content in these tissues, or because it is expressed in all tissues. The fact that we found only two of the cDNA clones out of eleven indicates that this alternative form is probably a minor species in brain and skeletal muscle (the strong intensity of the 312-bp band does not necessarily reflect the relative abundance of this form as the final PCR yield also depends on primer efficiency).

The alternative isoforms of dystrophin we have detected in the amino and carboxyl domains may not represent all possible forms of dystrophin in the cell as the PCR procedure can detect only those forms for which sequence is available. Analysis of cDNA clones from muscle and brain indicates that undetected forms are minor compared with those we present, otherwise they would have been isolated as cDNA clones. The first three domains of dystrophin are similar to those of the cytoskeletal proteins α -actinin and spectrin^{3,12,13} and the terminal 600 amino

acids of dystrophin have been subdivided into two domains on the basis of their cysteine content and similarity to α -actinin³. The variation in the primary sequence (Fig. 4) of dystrophin affects the last domain, which has no counterpart in α -actinin. It is interesting to speculate on the biological significance of the different forms of this domain in view of the surprising degree of conservation between the human and chicken dystrophin over the last 650 amino acids of the protein¹⁴, which implies that this terminal domain may interact with other highly conserved structural proteins. Here we have shown that this domain is alternatively spliced, resulting in a potential to encode different polypeptides. Each difference (Fig. 4) is generated by selective removal of exons at three key splice junctions. If the carboxyl domain anchors dystrophin to the inner surface of the plasma membrane through integral membrane proteins, then the discovery of tissue-specific isoforms indicates that proteins which interact with dystrophin could differ between tissues in which dystrophin is expressed. □

Received 19 December 1988; accepted 22 February 1989

- Hoffman, E. P., Brown, R. H. & Kunkel, L. M. *Cell* **51**, 919-928 (1987).
- Koenig, M. *et al. Cell* **50**, 509-517 (1987).
- Koenig, M., Monaco, A. P. & Kunkel, L. M. *Cell* **53**, 219-228 (1988).
- van Ommen, G. J. B. *et al. Genomics* **1**, 329-336 (1987).
- Burmeister, M. *et al. Genomics* **2**, 189-202 (1988).
- Saiki, R. K. *et al. Science* **239**, 487-491 (1988).
- Nudel, U. *et al. Nature* **337**, 76-78 (1989).
- Hoffman, E. P., Monaco, A. P., Feener, C. C. & Kunkel, L. M. *Science* **238**, 347-350 (1987).
- Lemaire, C., J. S. *et al. Science* **239**, 1416-1418 (1988).
- Hoffman, E. P., Hudecki, M. S., Rosenberg, P. A., Pollina, C. M. & Kunkel, L. M. *Neuron* **1**, 411-420 (1988).
- Chelly, J., Kaplan, J. C., Maire, P., Gautron, S. & Kahn, A. *Nature* **333**, 858-860 (1988).
- Hammond, R. G., Jr. *Cell* **51**, 1 (1987).
- Davison, M. D. & Critchley, D. R. *Cell* **52**, 159-160 (1988).
- Chamberlain, J. S. *et al. EMBO J.* **7**, 4157-4162 (1988).
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294 (1979).

ACKNOWLEDGEMENTS. We thank members of our laboratory, Alan Beggs, Rick Boyce, Eric Hoffman and Satori Iwamoto for critical reading of the manuscript, advice and unpublished information, and Rachael Neve for providing us with poly(A)⁺ RNA used in brain cDNA library construction. This work was supported by the Muscular Dystrophy Association (L.M.K.) and the National Institutes of Health. L.M.K. is an Associate Investigator of the Howard Hughes Medical Institute.

Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells

Daniel M. Altmann, Nancy Hogg*, John Trowsdale & David Wilkinson†

Human Immunogenetics Laboratory and *Macrophage Laboratory, Imperial Cancer Research Fund, PO Box 123, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

THE initiation of a specific immune response is believed to require not only activation through antigen-specific receptors on T cells and B cells but also antigen-independent interactions between accessory molecules¹. One such molecule is LFA-1, which enhances the avidity of interactions between T cells and antigen-presenting cells²⁻⁴, and is possibly involved in signal transduction across the T-cell membrane⁵. Intercellular adhesion molecule-1 (ICAM-1), a surface glycoprotein of relative molecular mass (M_r) 80,000–110,000, has been defined as a ligand for LFA-1^{6,7}, and has been shown to participate in the interaction between T cells and monocytes⁸. The determination of the precise contribution of such accessory molecules to antigen presentation, however, is complicated by the need to analyse against a background of multiple molecular interactions. We have investigated the role of LFA-1/ICAM-1 interactions in antigen presentation directly by quantifying the contribution of ICAM-1 expression to T-cell stimulation using L-cell transfectants that co-express ICAM-1 and HLA-DR. In the case of transfectants expressing modest levels of HLA-DR, co-expression of ICAM-1 is critical for effective HLA class II-restricted and allospecific T-cell activation, pointing to an important role for ICAM-1 in the induction of T-cell responses.

Previous studies from our laboratory showed that L-cell transfectants expressing a high level of HLA-DR2 could present antigen efficiently to several antigen-specific, HLA-DR2 restricted T-cell clones without any apparent requirement for LFA-1/ICAM-1 interactions⁹. Some T-cell clones, however, could not be activated in this way, implying that the expression of other human products by the antigen-presenting cells (APC), in addition to HLA class II, was required for effective antigen recognition. Moreover, using L cells transfected with HLA-DR7 (LDR7) (ref. 10), which express levels of HLA class II similar to monocytes, we have been unable to detect any significant antigen or allospecific stimulation of freshly isolated T cells. We therefore investigated whether reconstitution of APC function could be achieved in this case by supertransfecting the HLA-DR7 transfectants with the accessory molecule ICAM-1.

A full-length complementary DNA clone encoding ICAM-1 (ref. 7) was subcloned into the SV40-based cDNA expression vector pJ3 ω (ref. 11) and used to transfect mouse Ltk⁻ cells and L-cell transfectants expressing HLA-DR7 (LDR7) (ref. 10). After flow-microfluorometric sorting and single-cell cloning, cell lines expressing ICAM-1 (L-ICAM-1) and ICAM-1 plus DR7 (LDR7-ICAM-1) were obtained (Fig. 1). Both sets of transfectants bound the ICAM-1-specific monoclonal antibody RR1/1 (ref. 12), their level of expression being similar to that of the B-lymphoblastoid cell line PGF or fibronectin-adherent monocytes. Studies in murine systems have indicated that mouse L cells do not express their own functional ligand for mouse LFA-1 (ref. 13). The level of HLA class II expressed by the LDR7-ICAM-1 transfectants was indistinguishable from that of the parental cell line LDR7, this being $\sim 1/10$ that observed for the LDR2 transfectants. The large difference in the levels of

HLA class II expressed by LDR7 and LDR2 cells results from the use of different expression vectors during subcloning of the respective cDNA clones used for transfection^{9,10}.

Several studies have reported the failure of mouse L cells transfected with HLA products to serve as targets for alloreactive human cells^{14,15}. The transfectants L-ICAM-1, LDR7 and

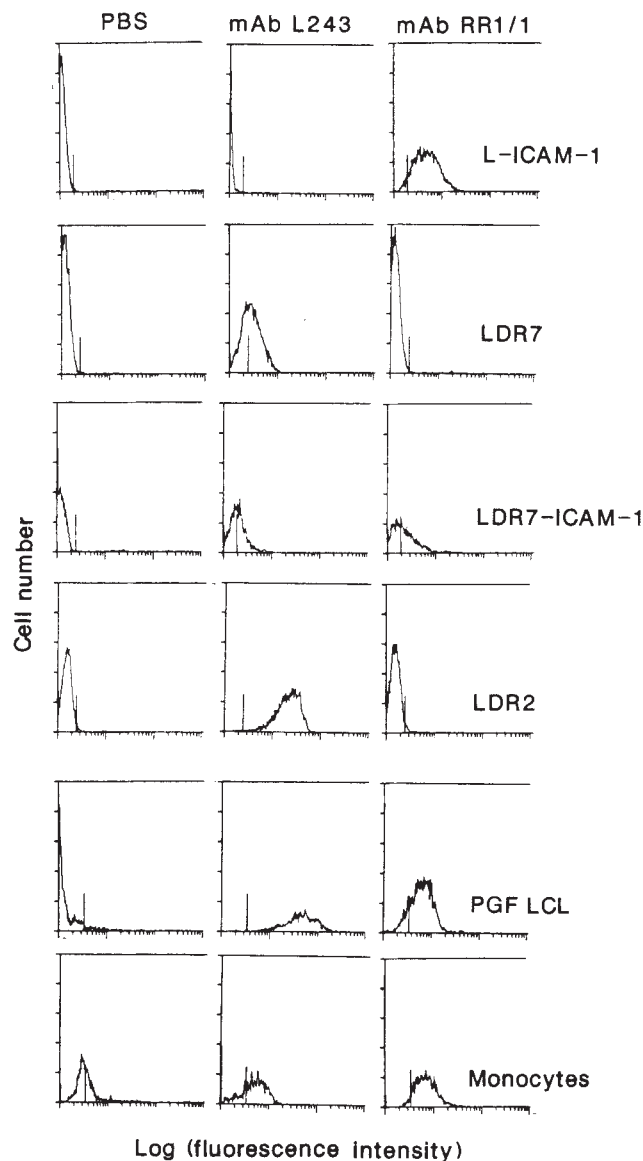


FIG. 1 Flow cytometric analysis of cellular HLA-DR and ICAM-1 expression. Cells were analysed for surface expression of HLA-DR (monoclonal L243) (ref. 17) and ICAM-1 (monoclonal RR1/1) (ref. 12), with rabbit anti-mouse immunoglobulin-FITC as second antibody, by flow cytometric analysis on a FACScan Analyzer (Becton Dickinson). The data are plotted as log fluorescence intensity (in arbitrary units) against cell number. Transfectants were compared with the HLA-DR2Dw2 homozygous B-lymphoblastoid cell line, PGF, and with peripheral blood monocytes prepared by overnight adherence of freshly isolated peripheral blood mononuclear cells to fibronectin-coated dishes. The level of HLA class II expression on monocytes varied among experiments and individuals; the data shown represent a population that falls within the limits of the observed range.

METHODS. Generation and characterization of the LDR7 and LDR2 transfectants has been described in detail elsewhere^{9,10}. L-ICAM-1 and LDR7-ICAM-1 transfectants were generated by transfection of Ltk⁻ cells and LDR7 cells respectively with an ICAM-1/pJ3 ω construct using the calcium phosphate precipitation technique. Plasmids encoding neomycin resistance (pSV2neo) or the herpes simplex virus thymidine kinase gene (pOPF) were cotransfected to allow selection of stable transfectants with Geneticin (Gibco) or hypoxanthine/methotrexate/thymidine, respectively. Drug-resistant colonies were pooled and established as stable lines before cloning.

† To whom correspondence should be addressed at the Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia

LDR7-ICAM-1 were therefore tested for cytotoxic allorecognition by JLMH1, a DR7-specific alloreactive T-cell line (Fig. 2a). At high effector/target ratios, the LDR7 transfectants showed only marginal specific lysis and no lysis of L-ICAM-1 cells was seen, indicating that neither molecule alone was sufficient to trigger significant effector function. The LDR7-ICAM-1 transfectants, however, served as efficient targets for JLMH1, the contrast with the targets expressing HLA-DR7 alone being most marked at low effector/target ratios. Cytolysis was inhibited by monoclonal antibodies (mAbs) against LFA-1 β (the common β -chain of the LFA-1 family¹⁶, ICAM-1 (ref. 12), HLA-DR (ref. 17) and CD4 (Leu3a) (Fig. 2b). Thus, the inefficient allorecognition

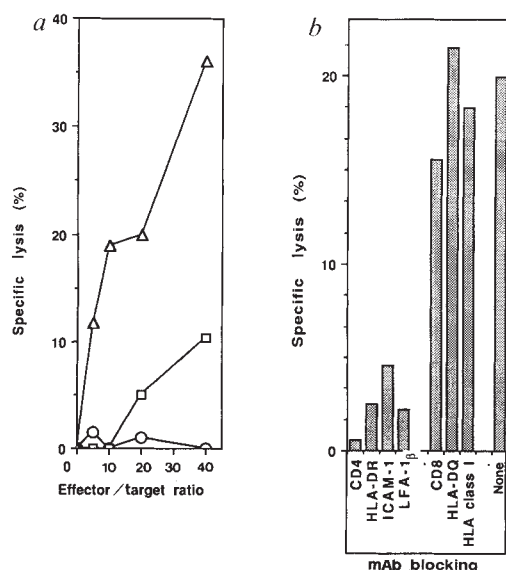


FIG. 2 Role of ICAM-1 in lysis of transfectants by cytotoxic T-cells. *a*, Target cells (1×10^4 per well) were labelled with ^{51}Cr (Amersham) and cultured at the indicated effector to target ratios with JLMH1 T-cells in V-bottom wells for 7 h, at which time supernatants were counted in a Beckmann Gamma 4000 Counter. Each point represents the mean of triplicate cultures. JLMH1 is a long-term, CD4⁺, alloreactive T-cell line, initially generated by stimulation of PBM cells from an HLA-DR4Dw4 homozygous individual with cells from an HLA-DR7 homozygous individual. The line is HLA-DR7-specific, recognizing all HLA-DR7 positive B lymphoblastoid cell lines but not HLA-DR1 or DR6 cell lines. Lysis of the HLA-DR7 homozygous B-LCL MANN was 83% (E/T = 40). Circles, L-ICAM-1; squares, LDR7; triangles, LDR7-ICAM-1. *b*, Cytolysis of the LDR7-ICAM-1 transfectant was assessed at an effector/target ratio of 20 in the presence of various mAbs. Antibodies were added to cultures as a 200-fold dilution of purified immunoglobulin or ascites representing a final concentration of $10\text{--}15 \mu\text{g ml}^{-1}$. Monoclonals used: Leu3a (anti-CD4); L243 (anti-DR)¹⁷; RR1/1 (anti-ICAM-1)¹²; MHM23 (anti-LFA-1 β chain)¹⁶; OKT8 (anti-CD8); Genox 3.53 (anti-HLA-DQw1); and W6/32 (anti-HLA class I).

of LDR7 was not due to the qualitative absence of a cell-type specific alloantigenic peptide, as has been suggested following the failure to observe allorecognition in other transfection systems^{15,18}, but rather reflects a quantitative dependence of T-cell recognition and/or effector function on accessory molecule interactions. Moreover, if allorecognition depends on low-affinity, cross-reactive recognition by T-cell receptors¹⁹, then accessory molecule interactions could make an important contribution to overall intercellular avidity.

A more stringent test of APC function is the presentation of antigen or alloantigen to freshly isolated peripheral blood mononuclear (PBM) cells. When the LDR7 transfectants were tested for the ability to stimulate alloreactive responses in HLA-mismatched PBM, they elicited no response (Fig. 3). In marked contrast, the LDR7-ICAM-1 transfectants stimulated large responses from non-HLA-DR7 (but not HLA-DR7) individuals, suggesting an important role for ICAM-1 in the induction of an alloreactive response against HLA-DR7 on these cells. But under identical conditions, the LDR2 transfectants, which express extremely high levels of HLA class II (Fig. 1), stimulated strong proliferative responses from non-HLA-DR2 PBM, without any requirement for ICAM-1 co-expression. These results provide evidence for a synergistic relationship between HLA class II and ICAM-1 in T-cell stimulation, the expression of ICAM-1 being of crucial importance under conditions of

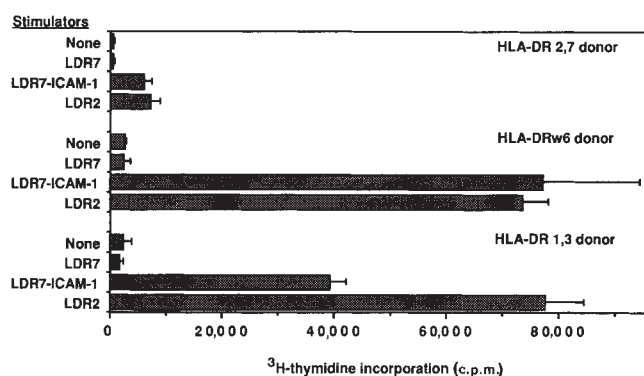
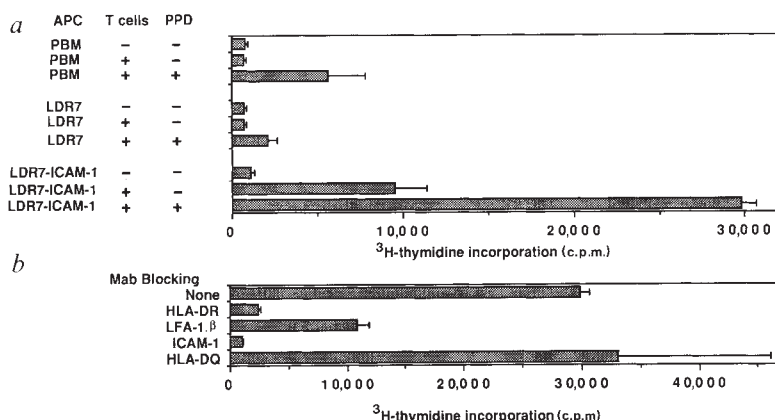


FIG. 3 Stimulation of freshly isolated peripheral blood mononuclear cells by allogeneic transfectants. Transfectants (5×10^4 per flat-bottom well) were treated with $100 \mu\text{g ml}^{-1}$ mitomycin c (Sigma) at 37° for 1 h before co-culturing in Iscove's modified Dulbecco's medium (Gibco Laboratories) with 1×10^5 freshly isolated peripheral blood mononuclear cells from individuals of the indicated HLA-DR types. Responses of donors to complete HLA haplotype-mismatched B-LCL ranged $105,000\text{ c.p.m.}$ – $182,000\text{ c.p.m.}$ Cells were cultured for 6 d, adding $1 \mu\text{Ci ml}^{-1}$ [^3H] thymidine (Amersham) for the final 6 h before collecting onto filter sheets and counting in a 1205 Betaplate scintillation counter (LKB). Bars represent the mean responses ($\pm$ s.e.) from triplicate cultures.

FIG. 4 Presentation of purified protein derivative (PPD) to freshly isolated T-cells by LDR7-ICAM-1 transfectants. *a*, Freshly isolated peripheral blood mononuclear cells from an HLA-DR2, 7 individual (1×10^5 per well, irradiated with 40 Gy), LDR7 transfectants or LDR7-ICAM-1 transfectants (treated as described in Fig. 3 legend) were tested for the ability to present PPD of *M. tuberculosis* (Statens Serum Institute, Denmark) added at a concentration of $25 \mu\text{g ml}^{-1}$ to autologous, nylon-wool-purified T-cells (1×10^5 per well). T-cells cultured with PPD in the absence of added APC gave a response of $1.168 \pm 485\text{ c.p.m.}$ Other details of culture and collecting are given in the legend to Fig. 3. Antibodies (details as in Fig. 2) were added to cultures containing purified T-cells, LDR7-ICAM-1 transfectants and PPD as in the lower part of panel *a*.



limiting HLA class II expression by the allogeneic stimulator. Similar synergistic effects between LFA-1 and class II have been reported in murine T-cell responses to antigen². Given that the absolute level of HLA class II expressed by the LDR7 transfectants is similar to that of activated monocytes, this dramatic effect of ICAM-1 on T-cell stimulation is probably of physiological relevance.

Alloreactive T-cells may differ in their activation requirements from antigen-specific, HLA-restricted T-cells. We therefore investigated the importance of ICAM-1 expression by the APC in recall antigen responses of freshly isolated T-cells. When purified T-cells from an HLA-DR7 responder were tested for their ability to respond to a purified protein derivative of *Mycobacterium tuberculosis* (PPD), a marginal response was observed using LDR7 cells as APC (Fig. 4a), which was amplified by co-expression of ICAM-1. Antigen presentation by the LDR7-ICAM-1 transfectants was inhibited by antibodies to HLA-DR, LFA-1 β , and ICAM-1 (Fig. 4b), confirming the HLA class II restriction and LFA-1/ICAM-1 dependence of the response. The LDR7-ICAM-1 transfectants also induced some T-cell proliferation in the absence of exogenous antigen or allogeneic HLA-DR (Fig. 4a). Indeed, L cells transfected with ICAM-1 alone delivered a small but significant mitogenic signal to purified T-cells which, although not as pronounced as the allorecognition demonstrable with LDR7-ICAM-1 transfectants, was significant compared with the response against control Ltk⁻ cells and was specifically inhibited by mAbs against ICAM-1 or LFA-1 α (data not shown). Although enhancement of a small xenogeneic response to mouse class I antigens cannot be discounted, these results suggest that the effects on recognition that follow transfection of ICAM-1 may involve not only enhanced conjugate formation with T-cells, but the delivery of activation signals through the ICAM-1/LFA-1 interaction itself. Such a pathway is implied by the T-cell mitogenic effect of some LFA-1 antibodies⁵.

Using DNA-mediated gene transfer we have been able to define the contribution of LFA-1/ICAM-1 interactions to antigen presentation in a manner not complicated by the expression of other APC-derived accessory molecules. In the absence of high levels of HLA class II expression by the APC, ICAM-1 expression can be a decisive factor in determining whether a T-cell response occurs. This observation is relevant not only to haemopoietic APC but also to cells of non-haemopoietic origin such as fibroblasts^{20,21}, endothelial cells, and epidermal keratinocytes^{22,23}, which express both HLA class II and ICAM-1 following exposure to interferon- γ , and may therefore serve as APC for self and foreign antigens²⁴. In addition, the profound effects of ICAM-1 expression on T-cell stimulation may have direct bearing on the relationship between the lack of expression of ICAM-1 (as well as other accessory molecules) on Epstein-Barr virus Burkitt's lymphoma lines and their inability to be recognized by anti-Epstein-Barr virus cytotoxic T-cells²⁵. □

21. Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A. & Springer, T. A. *J. Immun.* **137**, 245-255 (1986).
22. Racka, S. F., Charron, D. J. & Brodsky, F. M. *Hum. Immun.* **16**, 390-400 (1986).
23. Dustin, M. L., Singer, K. H., Tuck, D. T. & Springer, T. A. *J. exp. Med.* **167**, 1323-1340 (1988).
24. Bottazzo, G. F., Pujol-Borrell, R., Hanafusa, T. & Feldmann, M. *Lancet* **ii**, 1115-1117 (1983).
25. Gregory, C. D., Murray, R. J., Edwards, C. F. & Rickinson, A. B. *J. exp. Med.* **167**, 1811-1824 (1988).

ACKNOWLEDGEMENTS. We thank Drs David Simmons and Brian Seed for their ICAM-1 cDNA clone, Dr Tim Springer for RR1/1, Dr Andrew McMichael for MHM23 and MHM24, Dr J. Morgenstern for the vector pJ3 Ω , Mr Steven Marsh for HLA typing, and Dr Andrew Boyd for discussions.

Compliance of bacterial flagella measured with optical tweezers

Steven M. Block^{*†}, David F. Blair[†]
& Howard C. Berg^{*†}

^{*}Rowland Institute for Science, 100 Cambridge Parkway, Cambridge, Massachusetts 02142, USA

[†]Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138, USA

THE development of the gradient force optical particle trap ('optical tweezers') has made it possible to manipulate biological materials using a single beam of laser light¹. Optical traps can produce forces in the microdyne range on intact cells without causing overt damage: such forces are sufficient to arrest actively swimming bacteria² and can overcome torque generated by the flagellar motor of a bacterium tethered to a glass surface by a flagellar filament. By calibrating the trapping force against Stokes' drag and measuring the twist that is sustained by this force, we determined the torsional compliance of flagella in tethered *Escherichia coli* and a motile *Streptococcus*. Flagella behaved as linear torsion springs for roughly half a revolution, but became much more rigid when turned beyond this point in either direction.

Many motile bacteria are propelled by flagellar filaments, each of which is turned at its base by a reversible rotary motor driven by a transmembrane proton gradient³⁻⁵. In *E. coli*, the filament is a long helical polymer of a protein, flagellin, of molecular weight about 55,000. The filament is joined to the motor by means of a short hook (~80 nm long), a polymer of another protein, that is believed to serve as a flexible coupling^{3,5}. The filament, hook and motor (or basal structure) constitute the flagellum. Wild-type filaments are polymorphic: left- and right-handed forms have been described with a variety of helical pitches, depending on pH, ionic strength and torsional load⁵. Under normal physiological conditions, they assume a left-handed configuration, with wavelength ~2.5 μ m. When the motors turn anticlockwise (as viewed looking down the filament at the cell), the filaments move together to push the cell forward. When the motors turn clockwise, the filament bundle flies apart and the cell tumbles. Polymorphic transitions have been observed during tumbling which convert the filaments to right-handed forms with roughly half the wavelength⁶. When tethered to a surface by a single filament, a cell spins alternately clockwise and anticlockwise at nearly the same angular speed, changing direction abruptly and at random^{7,8}; some cells occasionally pause⁹. At room temperature, filaments in bundles spin at about 100 Hz, whereas tethered cell bodies spin at about 10 Hz. The motor exerts its maximum torque at stall, dropping monotonically with increased speed¹⁰.

Here we used cells whose motors had been paralysed by mutation, or de-energized by starvation or treatment with agents that dissipate the transmembrane proton gradient. These cells were tethered and spun by an external torque that was applied with an optical trap (Fig. 1). The central component is an infrared laser (wavelength 1,064 nm) whose beam is brought to a diffraction-limited focus by an objective of high numerical aperture. Transparent refractile materials such as bacteria experience forces that arise from induced dipole interactions

Received 9 January; accepted 16 February 1989.

1. Springer, T. A., Dustin, M. L., Kishimoto, T. K. & Marlin, S. D. *A. Rev. Immun.* **5**, 223-252 (1987).
2. Gougeon, M.-L., Bismuth, G. & Theze, J. *cell. Immun.* **95**, 75-83 (1985).
3. Dougherty, G. J. & Hogg, N. *Eur. J. Immun.* **17**, 943-947 (1987).
4. Shaw, S. *et al. Nature* **323**, 262-264 (1986).
5. van Noesel, C. *et al. Nature* **333**, 850-852 (1988).
6. Marlin, S. & Springer, T. A. *Cell* **51**, 813-819 (1987).
7. Simmons, D., Makgoba, M. W. & Seed, B. *Nature* **331**, 624-627 (1988).
8. Dougherty, G. J., Murdoch, S. & Hogg, N. *Eur. J. Immun.* **18**, 35-39 (1988).
9. Wilkinson, D. *et al. J. exp. Med.* **167**, 1442-1458 (1988).
10. Young, J. A. T., Wilkinson, D., Bodmer, W. F. & Trowsdale, J. *Proc. natn. Acad. Sci., U.S.A.* **84**, 4929-4933 (1987).
11. Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsburn, M. *Nature* **331**, 173-175 (1988).
12. Rothlein, R., Dustin, M. L., Marlin, S. D. & Springer, T. A. *J. Immun.* **137**, 1270-1274 (1986).
13. Golde, W. T. *et al. J. exp. Med.* **161**, 635-640 (1985).
14. Barbosa, J. A. *et al. Proc. natn. Acad. Sci., U.S.A.* **81**, 7549-7553 (1984).
15. Eckels, D. D., Sell, T. W., Long, E. O. & Sekaly, R. P. *Hum. Immun.* **21**, 173-181 (1988).
16. Hildreth, J. E. K., Gotch, F. M., Hildreth, P. D. K. & McMichael, A. J. *Eur. J. Immun.* **13**, 202-208 (1983).
17. Lampson, L. A. & Levy, R. J. *Immun.* **125**, 293-299 (1980).
18. Marrack, P. & Kappler, J. *Nature* **332**, 840-843 (1988).
19. Matis, L. A., Soergel, S. B., McElliot, D. L., Fink, P. J. & Hedrick, S. M. *Cell* **51**, 59-69 (1988).
20. Collins, T. *et al. Proc. natn. Acad. Sci., U.S.A.* **81**, 4917-4921 (1984).

with the light gradient, which tend to pull them into a trapping zone located just beyond the focus. The dimensions of the zone are comparable with the wavelength. Beam-steering optics allow the trap to be positioned manually in x , y , and z dimensions and swept electronically clockwise or anticlockwise in a circle in the x - y (specimen) plane. The laser is equipped with an attenuator to adjust the force and with a shutter to gate the trap.

At a low Reynold's number ($\sim 10^{-5}$ in the case of *E. coli*), the externally applied torque is balanced by torques arising from viscous drag on the cell body, torsion in the tether and thermal motion

$$f_{\theta}\Omega(t) + k_{\theta}\theta(t) + L(t) + N(t) = 0 \quad (1)$$

where f_{θ} is the rotational drag coefficient of the cell body, $\Omega(t)$ is its angular velocity, k_{θ} is the torsional spring constant of the tether, $\theta(t)$ is its twist, $L(t)$ is the Langevin torque arising from random bombardment by molecules in the medium, $N(t)$ is the applied torque and t is the time. Three kinds of behaviour can be distinguished, depending on the degree to which the motor resists rotation. (1) The motor turns freely, that is, it swivels about its tether and $k_{\theta}\theta$ vanishes. (2) The motor locks up partially, that is, it fails to turn until the torque arising from twist in the tether reaches a finite value, whereupon it slips; $k_{\theta}\theta$ has some upper bound. (3) The motor locks up entirely, that is, the cell body becomes clamped to the end of the tether, in which case $\Omega(t) = d\theta/dt$. Measurements on partially or fully locked cells enable k_{θ} to be determined. Note that for small angular deviations, the behaviour of partially or fully locked cells is the same.

E. coli carrying mutations in *motA* or *motB*, the genes implicated in torque production, have flagella that fail to rotate¹¹⁻¹³. We found that tethered cells in which either or both genes had been deleted diffused freely, as judged by videotape recordings and time-exposure photomicrographs; the time for a random excursion through 2π radians was typically several minutes, consistent with free Brownian rotation. When these cells were

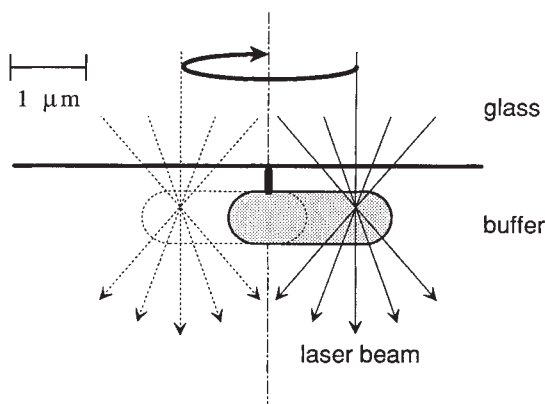
spun through several revolutions by the optical trap and then abruptly released by shuttering the laser, they stopped in place. The external torque required to rotate cells carrying the double deletion $\Delta(motA-motB)$ was proportional to the angular velocity (measured from 0.4×10^{-11} dyne-cm at 0.5 Hz to 2.6×10^{-11} dyne-cm at 3.0 Hz); least-squares fits passed through the origin.

Measurements of this kind were quantitated by calibrating the trap against Stokes' drag. A free cell was captured by the trap; this orientated its long axis parallel to the beam. The light level was fixed with the attenuator and the cell was moved along a circular trajectory ($\sim 4 \mu\text{m}$ in diameter). This path was retraced at increasing angular velocity until the transverse drag on the cell was just sufficient to pull it from the trap, that is, until the trapping force was balanced by Stokes' drag. This 'critical angular velocity' was recorded over the range of attenuator settings and a calibration curve constructed. As anticipated, this velocity (and hence the force) was strictly proportional to light level. The drag force was computed from the critical angular velocity, circumference of the trajectory, viscosity, and the transverse linear drag coefficient for that cell. To compute the drag coefficient, the cell's shape was approximated by a cylinder with hemispherical endcaps¹⁴; measurements of its dimensions were made from recordings using a computer-based video measurement system under conditions of negligible blooming¹⁵. Torques applied to tethered cells were computed from the product of the trapping force and the trap eccentricity (distance from centre of trap to centre of rotation). A correction factor equal to the aspect ratio of the cell was applied to the calibration to compensate for the optical path through the cell being shorter when its long axis was perpendicular to the laser beam. This correction gave excellent agreement between the measured slopes of the torque-velocity curves for $\Delta(motA-motB)$ cells and their computed rotational drag coefficients¹⁴.

Wild-type cells normally spin when tethered, but their motors

FIG. 1 Sketch of a bacterial cell being rotated about its tether by the optical trap. The axis of rotation of the trap (dashed line) was aligned with the tether (short thick line). The sense of rotation is shown by the circular arrow. Two views of the cell are displayed, at $\theta = 0^\circ$ (solid lines) and $\theta = 180^\circ$ (dotted lines). The laser beam enters from above, through the coverslip, as shown by the crossed rays. The microscope illumination (not shown) enters from below.

METHODS. Cells of *E. coli* strains RP437 (wild type for motility and chemotaxis), RP6665 ($\Delta(motA-motB)$), RP421 (*motB*⁵⁹⁰) and RP6894 ($\Delta(motA-motB)$) containing plasmid SYC62 encoding wild-type *motB* (gifts from S. Parkinson) were grown at 35 °C in tryptone broth, washed twice in buffer, shorn, washed twice again, and tethered as described²⁴, except that the buffer was 0.01 M potassium phosphate, pH 7.0, made 0.067 M in NaCl and 10^{-4} M in EDTA, and cover slips were cleaned with acetone followed by saturated KOH in ethanol. Cells of *Streptococcus* strain V4051 were grown at 35 °C in 1% K_2HPO_4 , 1% tryptone, 0.5% yeast extract, 1% glucose medium, washed twice in buffer, shorn, washed twice again, and tethered as described previously²⁵, except that the buffer used was 0.01 M TES, 0.01 M MES (pH 8.5), 0.2 M KCl, 10^{-4} M EDTA; cover slips were cleaned as above. The cover slips were mounted in a small flow cell²⁶. Experiments were at room temperature. *E. coli* were de-energized by flowing in buffer containing 2,4-dinitrophenol (DNP, 3 or 10 mM) for several minutes. *Streptococci* were de-energized by starvation for ≥ 30 min in buffer, or by treatment with buffer containing DNP (3 mM) or trifluoromethoxycarbonyl-cyanide phenylhydrazine (FCCP, $10 \mu\text{g ml}^{-1}$). To suppress possible photodynamic effects with DNP, a 1 cm-thick filter containing DNP (~ 10 mM) was interposed between the microscope lamp and the specimen. The optical trap comprised a Nd:YAG diode-pumped microlaser (Amoco Laser Co., ALC-1064, selected for 95 mW power output), a 3 $\times$ beam expander, a relay lens (CVI Laser, laser aplanat) held by a micropositioner, an infrared/visible dichroic mirror (CVI Laser) mounted in the epi-head of a research microscope (Zeiss, Universal), and a phase-contrast objective of high numerical aperture (Nikon, 60 $\times$ Planapo, DM series, 1.4 NA). The beam diameter was adjusted to fill the rear pupil of the objective. Light intensity was varied with a dual-wedge compensating attenuator (Newport, 925B) and gated by an electronic shutter (Uniblitz, 26L). Coarse x - y beam steering and z -focusing were achieved by adjusting the position of the relay lens with micrometer



screws. The relay lens was mounted at the end of a rigid cantilever; fine x - y steering was achieved by energizing coils that wrapped around this cantilever and extended through fields generated by permanent magnets. The steering system had a resonant frequency of 33 Hz and was critically damped with a dashpot filled with a halocarbon oil. The coils were driven by a quadrature oscillator that produced circular displacement of the lens; the direction, centre position, speed and amplitude of the circle were adjustable. The trap position was monitored by a charge-coupled device camera (Pulnix, TM-840N) that picked up the infrared reflection of the beam spot off the cover slip; the same camera was used to videotape all experiments. In control experiments designed to assess possible damage caused by high laser light levels, *Streptococcus* was tethered in growth medium and arrested with the beam at full power. The beam was gated off for a few seconds every minute to release the cell and allow it to spin. No reduction in motility was observed over a period of hours; indeed, the cell elongated and eventually divided. *E. coli* was far less resistant to light; cells lost motility after several minutes' exposure. Therefore, we used minimal light levels and kept the laser shuttered at all times when measurements were not being made.

can be arrested by treatment with protonophores such as 2,4-dinitrophenol (DNP) and trifluoromethoxycarbonyl-cyanide phenylhydrazine (FCCP). In the case of *Streptococcus*, motors can also be halted by starvation⁴. These treatments produced a range of phenotypes. In contrast with previous reports^{4,16}, we found that many of these cells were able to diffuse freely. In some cases this motion was hindered at certain fixed angles, probably as a result of interactions with the tethering surface. Other cells locked up, either partially, so that the cell could still be driven through many revolutions with the light trap, or entirely, so that it could not. When a partially locked cell was spun through several revolutions and then abruptly released, the cell body rebounded elastically through a fraction of a revolution (typically 60°). For example, in an experiment with starved *Streptococcus*, a cell was spun either clockwise or anticlockwise at 1.5 Hz for several seconds and then released at a random angle. The rebound angle was distributed normally with a mean and s.d. of $61^\circ \pm 27^\circ$ ($n = 108$). The amplitude of the recoil seemed to be independent of the direction of rotation and diminished by only 15% when the initial angular velocity was halved. There was no obvious spatial periodicity to the angular positions of the cell after rebounding. When we measured the external torques that were required to turn partially locked, DNP-treated cells of *E. coli* as a function of angular velocity, we obtained straight lines which were displaced upwards from the origin by $\sim 2 \times 10^{-12}$ dyne-cm. This corresponds to an additional torque required to overcome the barrier caused by the partial lock-up.

The lock-up behaviour of protonophore-treated *E. coli*, or of starved or protonophore-treated *Streptococcus*, implies the presence of an energy barrier to rotation which is larger than kT , where k is Boltzmann's constant and T is the absolute temperature. A typical record of jitter angle for *Streptococcus* is displayed in the inset to Fig. 2. By the equipartition theorem, $kT/2 = k_\theta \langle \theta^2 \rangle / 2$, permitting an estimate of k_θ directly from the mean-square angle¹⁶. For this cell, we obtained $k_\theta = 1.18 \times 10^{-12}$ dyne-cm rad⁻¹. The autocorrelation of the angle, $I(\tau)$, is obtained by solving equation (1) with $N(t) = 0$ and $\Omega(t) = d\theta/dt$, giving $I(\tau) = I_0 \exp(-|\alpha\tau|)$, where τ is the lag

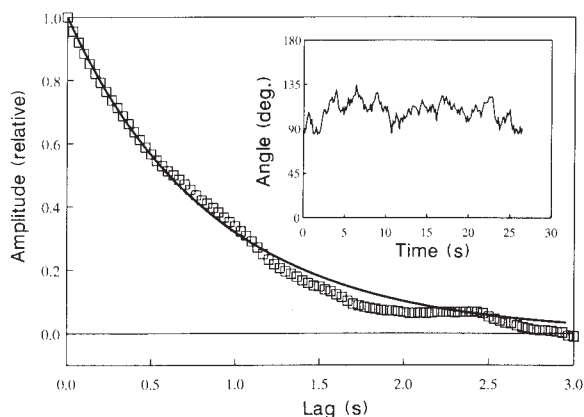


FIG. 2 Noise analysis of tethered *Streptococcus*. The graph shows the normalized autocorrelation function (squares) of angular jitter due to Brownian rotation of a partially locked *Streptococcus* cell treated with FCCP. The data were fitted by an exponential of time constant 0.875 s, solid line. Inset: The data from which the autocorrelation was computed, showing the angular position of this cell for 26.4 s. The root-mean square amplitude of the angular distribution was 10.7° .

METHODS. The autocorrelation as computed by standard fast-Fourier-transform technique²⁷ from an 800-point record of the angular position, with points digitized at every video-frame interval (1/30 s). Angles were measured with a computerized 'video protractor' coupled to an incremental shaft encoder (Vernitech, VEOL-23). Measurement error, determined from recordings of stuck cells, was $\pm 4^\circ$. Mean-square angle is a biased estimator, because zero-mean noise adds as a square, so values were corrected by subtracting the squared measurement error.

time and $\alpha = k_\theta/f_\theta$ (ref. 17). The experimental data are fitted by an exponential of time constant $\alpha^{-1} = 0.875$ s (Fig. 2). The rotational drag coefficient, f_θ , was estimated from the cell's geometry and the viscosity¹³, giving $k_\theta = \alpha f_\theta = \alpha f_\theta = 1.21 \times 10^{-12}$ dyne-cm rad⁻¹.

A more direct measurement of k_θ was made from the kinetics of the rebound that occurred when a partially locked cell was spun and released (Fig. 3). In this relaxation experiment, $N(t)$ is reduced from a fixed value to zero, and $\theta(t)$ decays as $\exp(-\alpha t)$ to zero. The upper curves display the angle as a function of time after release, using the same cell as shown in Fig. 2. The angle relaxed exponentially, as required by equation (1), with time constant $\alpha^{-1} = 0.745$, giving $k_\theta = \alpha f_\theta = 1.42 \times 10^{-12}$ dyne-cm rad⁻¹. This value is slightly larger than that obtained by noise analysis, in which a small amount of drift in the steady-state angular position would exaggerate $\langle \theta^2 \rangle$, leading to an underestimate of k_θ . The lower curves of Fig. 3 show analogous data for *E. coli*, with a time constant of $\alpha^{-1} = 0.143$ s, nearly five times faster, and giving $k_\theta = 4.03 \times 10^{-12}$ dyne-cm rad⁻¹. Cells of *E. coli* are smaller and their flagella appear to be more than twice as stiff, a property reflected in the small root-mean-square jitter observed in tethered *E. coli* ($\sim 6^\circ$) compared with *Streptococcus* ($\sim 11^\circ$).

A motor driving a tethered cell of *Streptococcus* generates a torque of $\sim 3 \times 10^{-11}$ dyne-cm¹⁰. Were the flagellum to behave as an ideal spring throughout its full range of movement with $k_\theta \approx 1.3 \times 10^{-12}$ dyne-cm rad⁻¹ (the value determined above), it would twist nearly four complete turns when subjected to this torque. Were the motor to reverse suddenly, the cell body would not follow suit until the flagellum had unwound four turns, and it would not reach top speed until the flagellum had wound up four turns in the opposite direction. Even if the motor were run at its maximum speed throughout this manoeuvre (100 Hz at room temperature¹⁰), the reversal would take roughly 0.1 s. Reversals are much more abrupt than this (< 0.01 s for *E. coli*¹⁶ and < 0.03 s for *Streptococcus*; S.M.B., D.F.B. and H.C.B., unpublished data).

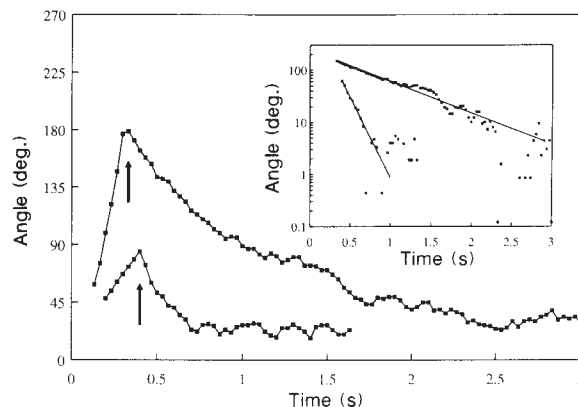


FIG. 3 Relaxation experiments with partially locked bacteria. The upper curve in the main plot shows the angle of the *Streptococcus* cell of Fig. 2 as a function of time, digitized at each video-frame interval. The cell was driven anticlockwise by the light trap and released at the time indicated by the arrow. The cell rebounded clockwise, relaxing to equilibrium. The lower curve shows a similar experiment with a wild-type cell of *E. coli* treated with 10 mM DNP. Inset: Semilog plots of these data from the time of the arrows, after subtraction of the baseline angle, and linefits, (solid lines) showing the exponential character of the relaxation. Time constants for decay and computed rotational drag coefficients: 0.745 s, 1.06×10^{-12} dyne-cm rad⁻¹ s⁻¹ and 0.143 s, 5.76×10^{-13} dyne-cm rad⁻¹ s⁻¹ for *Streptococcus* and *E. coli* respectively.

METHODS. The attenuator was set to produce negligible trapping force, the angular speed was set to 0.5 Hz, and the trap was aligned with a tethered cell, as illustrated in Fig. 1. The beam was then shuttered and the attenuator reduced to a setting that gave strong trapping. The shutter was opened and the cell spun at a fixed speed between 0.5 and 2 Hz for a few seconds, at which point the shutter was closed and the angular relaxation recorded.

We resolved this paradox by determining compliance over a large angular range. Figure 4 shows the torsion angle as a function of torque for cells whose motors appeared fully locked. In this domain, for sufficiently slow rotation, $N \approx -k_\theta \theta$. The compliance (slope) shows linear elastic behaviour through some 100° of twist (with a value of k_θ similar to that found above), and then it becomes more than an order of magnitude stiffer. Because of the nonlinearity, a tethered cell needs only to twist its flagellum about half a turn before it becomes rigid; therefore, a reversal could be completed within one turn, namely in ≤ 0.01 s. In a parallel experiment, a wild-type cell was arrested by gentle fixation with glutaraldehyde. The compliance was symmetric for both clockwise and anticlockwise rotations (Fig. 4, inset). The smaller asymptotic angle observed for this cell might reflect the difference in tether lengths, which can vary from cell to cell.

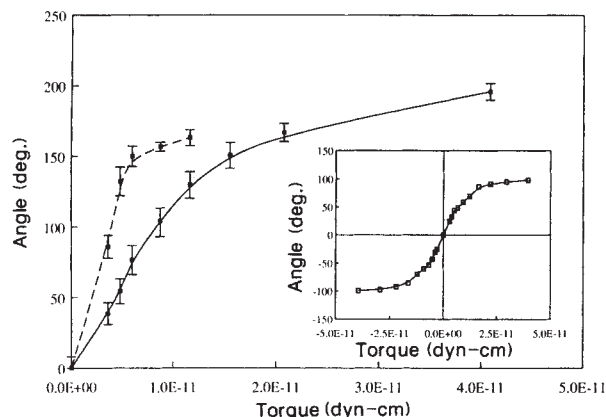


FIG. 4 Compliance curves for locked bacteria. The graph shows the angular displacement of fully locked cells as a function of the torque applied by the optical tweezers. Dashed line, data from a cell of *Streptococcus* treated with 10 mM DNP. Value for k_θ at small angles $\sim 2 \times 10^{-12}$ dyne-cm rad $^{-1}$. Solid line, data from a cell of *E. coli* treated with 10 mM DNP. Value for k_θ at small angles $\sim 5 \times 10^{-12}$ dyne-cm rad $^{-1}$. Error bars show the standard deviation for ten or more determinations of angle. Inset: Compliance curve for a cell of *E. coli* fixed with 0.1% glutaraldehyde in buffer, showing symmetry for clockwise and anticlockwise rotation.

METHODS. Cells which were apparently locked at a fixed angle were found by trial and error after treatment with 10 mM DNP. The trap was aligned as illustrated in Fig. 1, its speed was fixed at 0.25 Hz, and the eccentricity adjusted to maximize the torque. Then the beam was shuttered. The attenuator was reduced in a series of steps and the shutter opened for a short time at each level. As the trap encountered the cell body, it entrained it, turning it through part of a revolution until the trapping torque just balanced the elastic reaction of the tether. At this point, the cell escaped from the trap and rebounded to its original angle. This process was repeated at least ten times for each setting of the attenuator. Single-frame analysis with the video protractor provided the maximum angle attained for each trial. The force calibration curve and the trap eccentricity were used to convert the attenuator settings to torque. For most cells of *Streptococcus* (tethered without antibody), the highest values of torque would twist the cell off the tethering surface, terminating the experiment; this did not occur for cells of *E. coli* (tethered with antibody). In one instance involving *Streptococcus*, the cell remained well tethered, but the cell body suddenly stopped rebounding, remaining instead where it was when the beam was shuttered. Either the mechanism responsible for the lock-up was overcome or a component of the motor was broken. The torsion constant of a uniform hollow tube is related to the torsional rigidity, μ , by $k_\theta = \pi \mu (R^4 - r^4) / 2L$, where r and R are the inner and outer radii respectively, and L is the length²⁸. Using $R = 70$ Å and $r = 30$ Å, corresponding to the radii of maximum ('rod') density and of the inner hole, respectively, for filaments of *Salmonella typhimurium*²⁰, and our values of $L = 0.2$ μm and $k_\theta = 4 \times 10^{-12}$ dyne-cm rad $^{-1}$, we obtain $\mu = 2 \times 10^8$ dyne cm $^{-2}$, which is 3–4 orders of magnitude lower than an estimate¹⁹ (10^{11} – 10^{12} dyne cm $^{-2}$) derived from filaments of *S. typhimurium* that had been polymerized *in vitro*, but comparable to an estimate¹⁶ ($\sim 10^9$ dyne cm $^{-2}$) that was based on noise analysis of tethered *E. coli*. An explanation for this discrepancy could be that there is an additional compliance in flagella that is not present in filaments (see text and ref. 19). We note that the torsion constant attained by *E. coli* flagella at large angles is at least 1×10^{-10} dyne-cm rad $^{-1}$, implying that $\mu \geq 5 \times 10^9$ dyne cm $^{-2}$.

These lengths range from 0.1–0.5 μm, as estimated from the lateral displacements of cells that occur during normal reversals, or when cells are moved sideways with the optical trap. We note that both curves for *E. coli* in Fig. 4 are nearly superimposable when normalized to the maximum angle.

What is the explanation for the shape of the compliance curve? One possibility is that flagellar compliance consists of two distinct components: a 'soft' initial phase resulting from twisting of the proximal hook, and a 'hard' terminal phase dominated by the filament once the hook has reached some elastic limit. Published estimates of the flexural and torsional rigidity of isolated filaments^{18,19} indicate that they are far stiffer than our values of k_θ would imply (compare Fig. 4). It also is possible that the compliance is entirely dominated by the filament itself and that it is intrinsically nonlinear. A 0.2 μm-long tether carries roughly 80 turns of the lowest-order helical lattice, which has a pitch close to 26 Å and an outer diameter close to 200 Å (ref. 20). Twisting such a filament through one-half revolution would impose more than 2° of superhelical twist (shear) per turn, requiring a substantial accommodation of the structure. These possibilities could be distinguished by measuring the elastic properties of polyhook strains, which produce abnormally long hooks²¹, and/or by making compliance measurements over a wide range of filament lengths. We do not believe that the compliance is due to, say, elasticity of force generators which link the rotor to the cell wall, because DNP treatment also induced partial lock-up of $\Delta(motA-motB)$ cells. In relaxation experiments, these cells rebounded like wild-type cells. We did not see any abrupt changes in compliance which might be attributable to polymorphic transitions in the filament, even though the torques exceeded those experienced by flagella in tumbling cells, where such interconversions have been observed⁶. It is possible that interconversions did occur but remained undetected because of the shortness of the tethers, which are only a fraction of the normal flagellar wavelength. It is also possible that anti-filament antibody bound to the tether or tight association with the surface at the filament's distal tip prevented such cooperative transitions.

This elastic nonlinearity could be useful in motility. The filaments need to be stiff to function efficiently as propellers. But to form a coherent bundle, flagella emerging from distinct motors distributed over all parts of the cell body must coalesce, with attendant distortion of their helical geometry²². Early events in bundle formation may take advantage of a relatively soft (hook-based?) compliance to stabilize the coordinated bundle. We note that the torque produced by motors in actively swimming *Streptococcus* ($\sim 1 \times 10^{-11}$ dyne cm 10) is just sufficient to wind flagella beyond the linear compliance domain.

Optical tweezers provide a novel means of measuring the elastic properties of individual bacterial flagella, means that are hard to duplicate with conventional techniques, for example, those using thin glass needles²³. We anticipate that trapping technology will be applicable to the manipulation of a wide range of macromolecular assemblies. □

Received 16 January; accepted 13 February 1989.

1. Ashkin, A. & Dziedzic, J. M. *Science* **235**, 1517–1520 (1987).
2. Ashkin, A., Dziedzic, J. M. & Yamane, T. *Nature* **330**, 769–771 (1987).
3. Berg, H. C. & Anderson, R. A. *Nature* **245**, 380–382 (1973).
4. Berg, H. C., Manson, M. D. & Conley, M. P. *Symp. Soc. exp. Biol.* **35**, 1–31 (1982).
5. Macnab, R. M. in *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology* Vol. 1 (eds Neidhardt, F. C. et al.) 70–83, 732–759 (American Society for Microbiology, Washington DC, 1987).
6. Macnab, R. M. & Ornston, M. K. *J. molec. Biol.* **112**, 1–30 (1977).
7. Silverman, M. & Simon, M. *Nature* **249**, 73–74 (1974).
8. Berg, H. C. *Nature* **249**, 77–79 (1974).
9. Lapidus, I. R., Welch, M. & Eisenbach, M. *J. Bact.* **170**, 3627–3632 (1988).
10. Lowe, G., Meister, M. & Berg, H. C. *Nature* **325**, 637–640 (1987).
11. Silverman, M., Matsumura, P. & Simon, M. *Proc. natn Acad. Sci. U.S.A.* **73**, 3126–3130 (1976).
12. Block, S. M. & Berg, H. C. *Nature* **309**, 470–472 (1984).
13. Blair, D. F. & Berg, H. C. *Science* **242**, 1678–1681 (1988).
14. Meister, M. & Berg, H. C. *Biophys. J.* **52**, 413–419 (1987).
15. Sheetz, M. P., Block, S. M. & Spudis, J. A. *Meth. Enzym.* **134**, 531–544 (1987).
16. Berg, H. C. in *Cell Motility* Vol. A (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 47–56 (Cold Spring Harbor Press, New York, 1976).

17. Wang, C. W. & Uhlenbeck, G. E. in *Selected Papers on Noise and Stochastic Processes* (ed. Wax, N.) 113–132 (Dover, New York, 1954).
18. Fujime, S., Maruyama, M. & Asakura, S. *J. molec. Biol.* **68**, 347–359 (1972).
19. Hoshikawa, H. & Kamiya, R. *Biophys. Chem.* **22**, 159–166 (1985).
20. Trachtenberg, S. & DeRosier, D. J. *J. molec. Biol.* **195**, 581–601 (1987).
21. Silverman, M. R. & Simon, M. I. *J. Bact.* **112**, 986–993 (1972).
22. Macnab, R. M. *Proc. natn Acad. Sci. U.S.A.* **74**, 221–225 (1977).
23. Nicklas, B. A. *Rev. Biophys. biophys. Chem.* **17**, 341–449 (1988).
24. Block, S. M., Segall, J. E. & Berg, H. C. *Cell* **31**, 215–226 (1982).
25. Manson, M. D., Tedesco, P. M. & Berg, H. C. *J. molec. Biol.* **138**, 541–561 (1980).
26. Berg, H. C. & Block, S. M. *J. gen. Microbiol.* **130**, 2915–2920 (1984).
27. Press, W. H. et al. in *Numerical Recipes in C: The Art of Scientific Computing* Ch. 12 (Cambridge University Press, 1988).
28. Landau, L. D. & Lifshitz, E. M. *Theory of Elasticity* 2nd edn 68–75 (Pergamon, Oxford, 1970).

ACKNOWLEDGEMENTS. We thank S. Chu for advice, P. Horowitz for the scanning stage from his X-ray microscope, and M. M. Burns, J. A. Golovchenko and E. M. Purcell for comments on the manuscript. D.F.B. is a Burroughs Wellcome Fellow of the Life Sciences Research Foundation. This work was supported by the Rowland Institute for Science.

Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor

Thomas J. Girard*, Louise A. Warren*, William F. Novotny*, Karen M. Likert*, Susan G. Brown*, Joseph P. Miletich† & George J. Broze, Jr*‡

* Division of Hematology/Oncology, Jewish Hospital at Washington University Medical Center, 216 South Kingshighway Boulevard, St Louis, Missouri 63110, USA

† Department of Laboratory Medicine, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, Missouri 63110, USA

‡ To whom correspondence should be addressed.

BLOOD coagulation can be initiated when factor VII or VIIa, a plasma protease, binds to its essential cofactor, tissue factor (TF), and proteolytically activates factors IX and X^{1,2}, triggering a cascade of events which eventually leads to the formation of thrombin and a fibrin clot. Plasma contains a lipoprotein-associated coagulation inhibitor (LACI) which inhibits activated factor X (Xa) directly and, in a Xa-dependent way, inhibits VII(a)/TF

activity, presumably by forming a quaternary Xa/LACI/VII(a)/TF complex^{3–5}. Sequence analysis of complementary DNA clones has shown that LACI contains three tandemly repeated Kunitz-type serine protease inhibitory domains^{6,7}. To investigate the relationship between these Kunitz structures and LACI function, we have used site-directed mutagenesis to produce altered forms of LACI in which the residue at the active-site cleft of each Kunitz domain has been individually changed. The second Kunitz domain is required for efficient binding and inhibition of Xa, and both Kunitz domains 1 and 2 are required for the inhibition of VIIa/TF activity; but alteration of the active-site residue of the third Kunitz domain has no significant effect on either function. We propose that in the putative inhibitory complex, Kunitz domain 1 is bound to the active site of VII(a)/TF and that Kunitz domain 2 is bound to Xa's active site.

Bovine pancreatic trypsin inhibitor (BPTI, aprotinin) is the best characterized Kunitz-type inhibitor and alteration of the residue in the P1 position⁸ of the active-site cleft of BPTI profoundly alters its inhibitory activity⁹. By sequence homology alignment with BPTI, the P1 position for each of LACI's Kunitz domains was identified (ref. 6; Fig. 1). We used site-directed mutagenesis to prepare LACI mutants in which this active-site-cleft residue in each Kunitz domain has been individually changed. Thus, the mutants were designed to maximize the effect on each domain's function while minimizing the possible alteration in the protein structure. These modified LACI DNAs were introduced into the bovine papilloma virus vector pMON1123 and cotransfected with pSV2neo into mouse C127 fibroblasts.

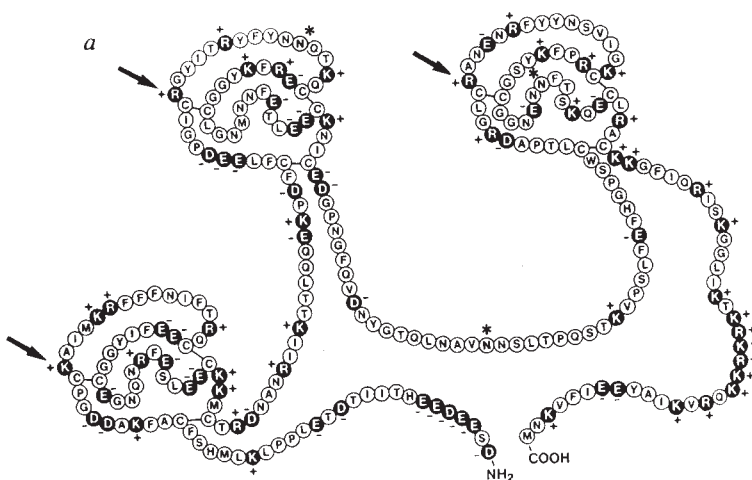
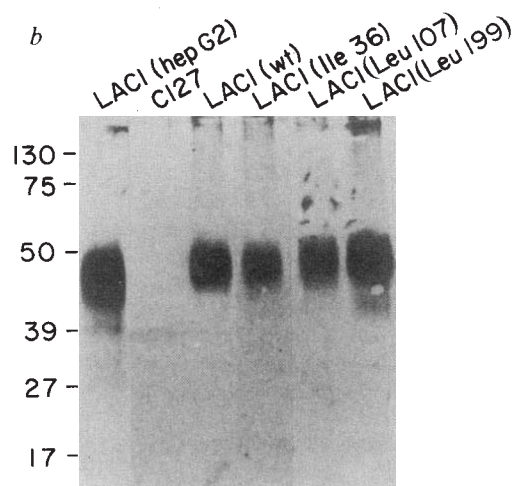


FIG. 1 *a*, Schematic diagram of LACI's predicted secondary structure showing the sulphhydryl bonding for the three Kunitz domains. The Kunitz domains are numbered from the one closest to the N terminus. The arrows indicate the location of the presumed P1 residues of the active-site clefts for the Kunitz domains^{8,9}. The charges of the amino-acid side chains are indicated (histidine side chains are considered as uncharged). Asterisks show the potential sites for N-linked glycosylation. *b*, Western blot showing the expression of the recombinant wild-type and mutant LACIs. Serum-free conditioned media (100 µl) from the indicated cells and 20 ng purified LACI(hepG2) were run on a 15% polyacrylamide gel, transferred to nitrocellulose, probed with mouse polyclonal anti-LACI antibodies and colourimetrically developed^{7,10}. Relative molecular masses are shown on the left in thousands.



METHODS. A modified LACI cDNA insert engineered into a bovine papilloma virus vector for expression in C127 fibroblasts⁷ was ligated into M13mp18 and site-directed mutagenesis¹¹ was performed as follows (the numbering of nucleotides and amino acids is according to the 4.0 kilobase (kb) LACI cDNA sequence⁷. LACI(Ile 36): A → T at 572 which changes Lys 36 to Ile. LACI(Leu 107) G → T at 785 which changes Arg 107 to Leu. LACI(Leu 199): G → T at 1,061 which changes Arg 199 to Leu. Sequences of the mutant molecules were confirmed by the dideoxy chain-termination procedure¹² and cloned into the bovine papilloma virus expression vector pMON1123 (ref. 7). Each of these expression vectors plus pSV2neo were cotransfected into mouse C127 fibroblasts using calcium phosphate precipitation³ and G418-resistant clones were screened for expression of LACI. LACI(hepG2) was purified as previously described⁴.

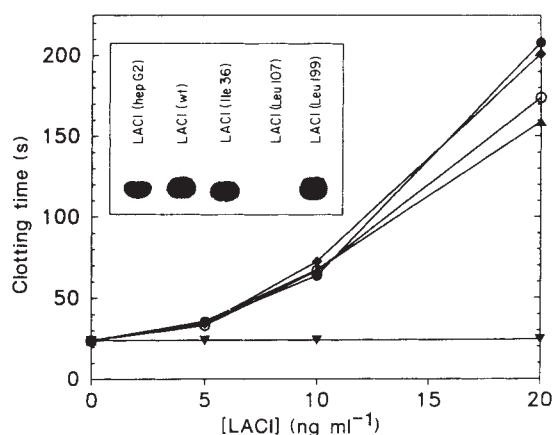


FIG. 2 Binding and inhibition of factor Xa by wild-type and mutant LACIs. Factor Xa inhibitory activities were determined as previously described³ using 25 ng ml⁻¹ (final concentration) bovine factor Xa. Samples are: LACI(hepG2) (○); LACI(wt) (●); LACI(Ile 36) (▲); LACI(Leu 107) (▼); LACI(Leu 199) (◆). Inset, ¹²⁵I-labelled Xa blot analysis of LACI mutants. Samples are designated in the same way as in Fig. 1.

METHODS. LACIs were precipitated from transfected cell clones' serum-free conditioned media using CdCl₂, resuspended in 0.25 M EDTA, pH 9.0, clarified and dialysed into 0.1 M NaCl, 0.05 M Tris-HCl, pH 7.5 (TS buffer)¹⁴. Recombinant LACIs were purified using a mouse monoclonal anti-LACI-affigel 10 column. Dialysed CdCl₂-concentrated LACI samples were applied to the column in TS, the column was washed with several volumes of TS and LACI was eluted with 2 M NaSCN. After the addition of BSA as carrier, concentration and dialysis into TS, the LACI concentration for each sample was determined in a particle-concentration immunofluorescence assay¹⁵ using two non-competitive anti-LACI monoclonal antibodies. For the ligand blot, 50 ng of each sample was electrophoretically fractionated by SDS-PAGE, transferred to nitrocellulose, and then probed with ¹²⁵I-labelled Xa (refs 7, 10). Western blot analysis using rabbit polyclonal anti-LACI antibodies was performed on a duplicate blot to confirm that equivalent amounts of LACI were present in each sample (data not shown).

Individual clones expressing human LACI messenger RNA (data not shown) and LACI protein were identified (Fig. 1) and recombinant LACIs were isolated from the clones' conditioned media using monoclonal anti-LACI affinity chromatography.

LACI isolated from the conditioned media of a human hepatoma cell line (hepG2), as well as that isolated from plasma, bind ¹²⁵I-labelled factor Xa on ligand blots and inhibit the enzymatic activity of factor Xa (refs 3 and 10; W.F.N. *et al.*, manuscript submitted). We assessed the ability of the modified LACI molecules to bind to and inhibit factor Xa. LACI(Ile 36) and LACI(Leu 199) with an altered active-site residue for Kunitz domains 1 and 3, respectively, both bound ¹²⁵I-labelled Xa on ligand blots and inhibited factor Xa to the same degree as recombinant wild-type LACI LACI(wt) or LACI(hepG2). But LACI(Leu 107), which contains an altered active-site residue in the second Kunitz domain, was not recognized by ¹²⁵I-labelled Xa, nor did it inhibit factor Xa (Fig. 2). These results indicate that the second Kunitz domain is responsible for the binding to, and inhibition of, factor Xa by LACI.

The ability of the altered LACI molecules to inhibit VII(a)/TF activity in the presence of Xa was determined using two separate assays. In the first, VII(a)/TF activity was determined by the release of the activation peptide from its substrate factor IX (ref. 7). LACI(Leu 199), which contains an altered Kunitz domain 3, inhibited the VII(a)/TF activity to the same degree as LACI(wt) and LACI(hepG2) (Fig. 3). Modification of either the first Kunitz domain, LACI(Ile 36), or the second Kunitz domain, LACI(Leu 107), did result, however, in the loss of VII(a)/TF inhibitory activity.

Similar results were obtained for the mutant proteins in a three-stage clotting assay which is dependent on VII(a)/TF activation of its other substrate, factor X. In this assay, inhibition of VII(a)/TF activity by LACI depends on the presence of factor X (which is converted to Xa) in the first stage of the assay³. Inhibition of VII(a)/TF activity in the first stage causes less factor X to be activated in the second stage, so clotting takes a

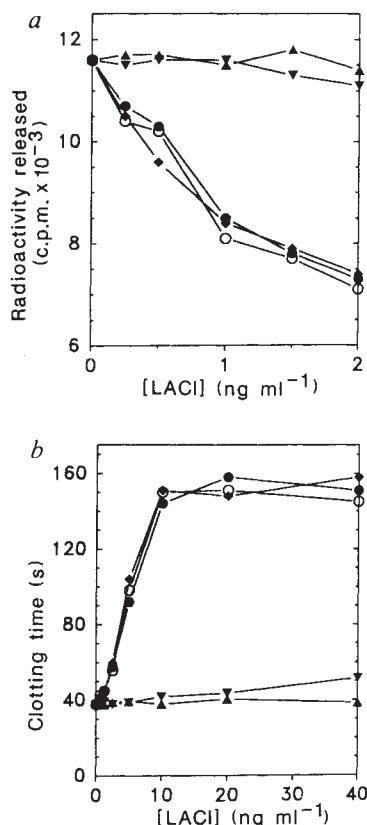


FIG. 3 VII(a)/TF inhibitory activities of the wild-type and mutant LACIs measured by a [³H] IX activation peptide release assay (a) and a three-stage clotting assay (b). Both assays were performed as previously described^{3,7} with LACI(hepG2) (○); LACI(wt) (●); LACI(Ile 36) (▲); LACI(Leu 107) (▼); LACI(Leu 199) (◆).

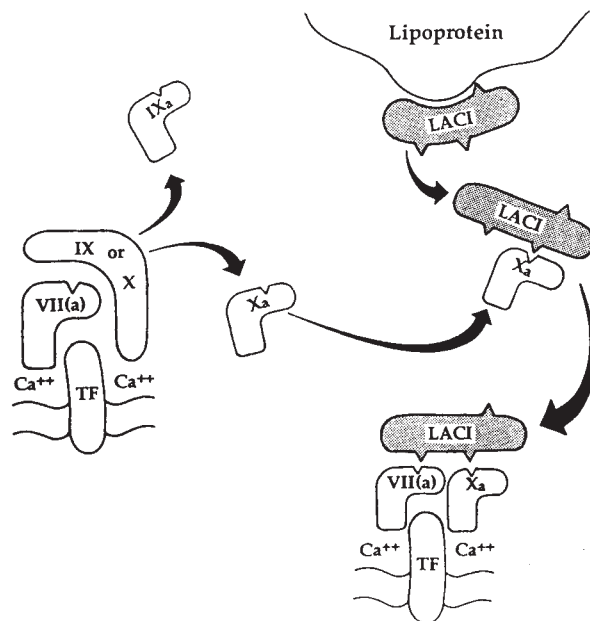


FIG. 4 Schematic diagram of the proposed mechanism for the inhibition of factor Xa and the VII(a)/TF complex by LACI. VII(a) denotes either VII or VIIa; the indentations represent the active sites for VII(a) and Xa; and the protrusions represent the three Kunitz domains of LACI. In the Xa/LACI complex, the active site of Xa is bound to the second Kunitz domain of LACI. In the final quaternary Xa/LACI/VII(a)/TF complex Xa is bound at its active site to LACI's second Kunitz domain and VII(a) is bound at its active site to the first Kunitz domain of LACI. The mechanism of the association of LACI with lipoproteins is not known.

longer time. Again, mutation of the active-site residue in either the first or second Kunitz domain abrogated LACI's ability to inhibit VII(a)/TF activity effectively, whereas mutation of the third Kunitz domain had no effect on activity. At high concentrations of LACI(Leu 107) a slight but reproducible inhibition was observed (Fig. 3). This low level of inhibition was also dependent on the presence of Xa in the first stage of the assay (data not shown).

Our current working hypothesis for the mechanism by which LACI inhibits VII(a)/TF activity is schematically shown in Fig. 4. When plasma is exposed to TF, VII(a) binds to TF and activates factors IX and X. Some of the Xa generated becomes bound by its active site to LACI's second Kunitz domain and is inhibited. This Xa/LACI complex then binds to, and inhibits, the VII(a)/TF complex, presumably by forming a Xa/LACI/VII(a)/TF quaternary complex. Alternatively, LACI may bind to a preformed VII(a)/TF/Xa complex³. We have previously shown that the N-terminal domain of Xa, containing its γ -carboxylglutamic acids which are required for Ca^{2+} binding, is necessary for inhibition of VII(a)/TF activity by the LACI/Xa complex³. The results presented here indicate that the second Kunitz domain is necessary for the formation of the Xa/LACI complex; this, and also the first Kunitz domain, are required for VII(a)/TF inhibition. The effect of domain 1 is presumably

mediated through its binding to the active site of VII(a). The native P1, residue of LACI's third Kunitz domain, however, does not seem to be required for these functions. It is conceivable that changing Arg 199 to Leu was not sufficient to affect the functional activity of the third domain, or that a portion of this domain unrelated to its active-site cleft is required for function, but we believe that both these possibilities are unlikely. $\square$

Received 19 December 1988; accepted 22 February 1989.

1. Silverberg, S. A., Nemerson, Y. & Zur, M. *J. biol. Chem.* **252**, 8481–8488 (1977).
2. Zur, M. & Nemerson, Y. *J. biol. Chem.* **255**, 5703–5707 (1980).
3. Broze, G. J. Jr et al. *Blood* **71**, 335–343 (1988).
4. Sanders, N. L., Bajaj, S. P., Zivelin, A. & Rapaport, S. I. *Blood* **66**, 204–212 (1985).
5. Hubbard, A. R. & Jennings, C. A. *Thromb. Res.* **46**, 527–537 (1987).
6. Wun, T.-C., Kretzmer, K. K., Girard, T. J., Miletich, J. P. & Broze, G. J. Jr *J. biol. Chem.* **263**, 6001–6004 (1988).
7. Girard, T. J. et al. *Nucleic Acids Res.*, submitted.
8. Berger, A. & Schechter, I. *Phil. Trans. R. Soc. B* **257**, 249–264 (1970).
9. Wenzel, H. R. & Tschesche, H. *Angew. Chem. Int. Ed. Engl.* **20**, 295–296 (1981).
10. Novotny, W. F., Girard, T. J., Miletich, J. P. & Broze, G. J. Jr *Blood* **72**, 2020–2025 (1988).
11. Zoller, M. J. & Smith, M. *Meth. Enzym.* **100**, 468–500 (1983).
12. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6776–6780 (1977).
13. Howley, P. M., Sarver, N. & Law, M. F. *Meth. Enzym.* **101**, 387–403 (1983).
14. Broze, G. J. Jr, Warren, L. A., Girard, T. J. & Miletich, J. P. *Thromb. Res.* **48**, 253–259 (1987).
15. Miletich, J. P., Sherman, L. & Broze, G. J. Jr *New Engl. J. Med.* **317**, 991–996 (1987).

ACKNOWLEDGMENTS We thank John Sneller for technical assistance and Diana Horn for help in the preparation of this manuscript.

Conformational dynamics of individual DNA molecules during gel electrophoresis

David C. Schwartz & Michael Koval

Carnegie Institution of Washington, Department of Embryology,
115 West University Parkway, Baltimore, Maryland 21210, USA

GEL electrophoresis is widely used in molecular biology to separate DNA molecules according to their sizes. The physical basis of this size separation is, however, poorly understood. Here we report observations of individual, fluorescently stained DNA molecules as they migrate during various kinds of gel electrophoresis. Their movement, under the influence of either a steady electric field or a pulsed-field, is characterized by cycles of elongation and contraction. Initially relaxed coils of DNA lengthen into 'hook-shaped' configurations which temporarily 'hang-up' on obstacles in the gel matrix before sliding off, contracting and entering another cycle. The effects of a new electrophoresis technique, termed 'pulse-oriented electrophoresis', which allows the effective angle of the electric field, and hence the molecular orientation of DNA, to be varied without electrode rearrangement, are also studied. In this case the DNA adopts a 'staircase' configuration showing that the net orientation in a direction is given by the vector sum of the pulses used.

Individual DNA molecules undergoing conventional gel electrophoresis were visualized using a microscope-mounted miniature electrophoresis apparatus. As shown in Fig. 1a–f, molecules initially in a random conformation elongated to form 'hook' structures which aligned with the applied electric field and 'wrapped around' obstacles in the gel. Tension between the two arms of a hook was resolved when one arm became dominant (Fig. 1d, e; molecule 1), pulling the other arm past the obstacle and facilitating coil relaxation to a more random conformation. Recurring cycles of elongation, resolution and relaxation were observed for the duration of the applied steady field. Collectively, these orientational events confirm previously published models^{3–6}. The application of a second electric field, oriented at 90° to the first, resulted in a realignment of DNA molecules (Fig. 1g–i). Immediately after field switching, segments of all

molecules began to move in the direction of the new field, and after 39 s, most of the molecules assumed hook structures that were somewhat broader than those found in the steady field case.

Separation by pulse-field electrophoresis depends on the angle between successively applied fields (field angle)^{7–11}, with obtuse angles providing superior separation of large DNA molecules. A new electrophoretic effect and its associated method, pulse oriented electrophoresis (POE), was thus used to study the relationship between field angle and DNA conformational changes.

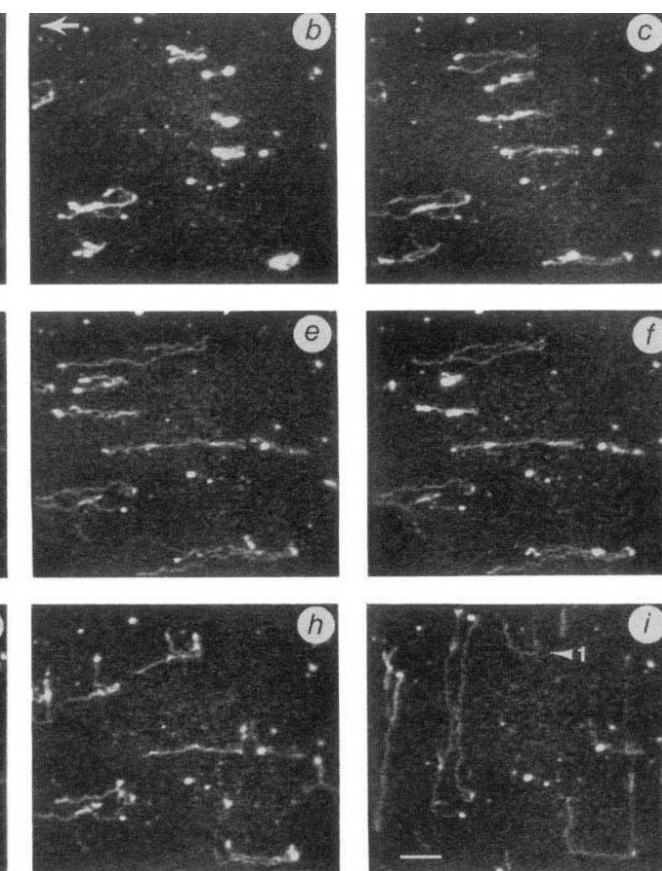
POE has been used to resolve the chromosomes of both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* in a manner analogous to the pulse electrophoretic method¹². Briefly, POE allows molecular orientation to be varied, without the requirement of electrode rearrangement, by using a series of alternating perpendicular pulses shorter than the orientation time for a given molecule. These pulses are applied such that their predicted effect is to fold a DNA molecule into a staircase-like configuration⁹. The net alignment of each rapidly pulsed molecule is then the vector sum of the DNA segmental orientations corresponding to the 'steps' of the staircase. This alignment can be altered by varying the pulse duration ratio of the two perpendicular fields. Thus, POE emulation of pulse-field electrophoresis using a 90° field angle (Fig. 2a–f) caused molecules to adopt the predicted 'staircase' conformation and exhibit coil dynamics similar to those shown in Fig. 1.

The emulation of an obtuse field angle (~120°) by POE (Fig. 3) resulted in molecules elongating to form broad hooks which also exhibited the staircase effect. These structures collapsed within 6 s of switching fields, whereas roughly 20 s were required for collapse after a 90° field switch (Fig. 2); the apparent coil relaxation time after electric field cessation, however, was about 60 seconds (Fig. 4).

Our results suggest a model for the field-angle-dependent reorientation mechanism of POE that might also apply to pulse-field electrophoresis. Consider the electric field force components generated along a stretched DNA coil axis (constrained by the gel matrix) immediately after the field direction has shifted. If the field angle is 90°, then, on average, both ends of an extended coil will be equally favoured to move in the direction of the new electric field and this inhibits coil relaxation. If the field angle is obtuse, however, then the new electric field will have a component parallel to the elongated coil axis which can

FIG. 1 G bacteriophage DNA molecules, 700 kilobases (kb) long, stained with 4,6-diamidino-2-phenylindole (DAPI) and visualized during gel electrophoresis. The images were obtained at 0 s (a), 5 s (b), 16 s (c), 28 s (d), 33 s (e), 44 s (f), 54 s (g), 60 s (h) and 93 s (i) after field initiation. a, The apparent radius of relaxed G bacteriophage DNA (see arrowheads) in a 1.0% low-melting-temperature gel matrix; this was similar to the solution value of 2.2×10^{-4} cm. b, The effect of a uniform electric field (3.5 V cm^{-1}); the coils move and elongate in the direction of the applied field (arrow). b–f, The continuing movement produces a series of hook structures and thick rod-like coil conformations. g–i, The effect of shifting the field direction by 90° (arrow); the molecules reorient. i, Bar; $10 \mu\text{m}$.

METHODS. G bacteriophage was grown as previously described⁴. DNA was prepared by lysing virus in $1/2 \times \text{TBE}$ buffer (42.5 mM Trizma base, 44.5 mM boric acid, 1.25 mM disodium EDTA) followed by ethanol precipitation; this did not shear the sample. DNA in $1/2 \times \text{TBE}$ was diluted to $0.1\text{--}0.2 \text{ ng ml}^{-1}$ in prefiltered 1.0% low-gelling-temperature agarose (Sea Plaque) in $1/2 \times \text{TBE}$, $0.3 \mu\text{g ml}^{-1}$ DAPI (Sigma), 1.0% 2-mercaptoethanol and held at 65°C . Samples were mounted using $3 \mu\text{l}$ DNA-agarose mixture, transferred to a preheated slide, covered, then placed into a miniature pulse electrophoresis apparatus consisting of a series of discrete platinum electrodes surrounding the square cover slip, wetted with agarose for contact, and connected to diodes to prevent electrode-generated field distortion effects. Samples were pre-electrophoresed for 2–3 min and allowed to relax at room temperature before observation. Field strength was measured using auxiliary electrodes connected to a multimeter. A



Zeiss Axioplan microscope and a C2400-SIT camera (Hamamatsu Corp.) were used with low level excitation light to minimize photodamage during imaging. For each image, eight video frames were digitized, boxcar-averaged and corrected to remove background fluorescence using an IC-1 image processing system (Inovision Corp.).

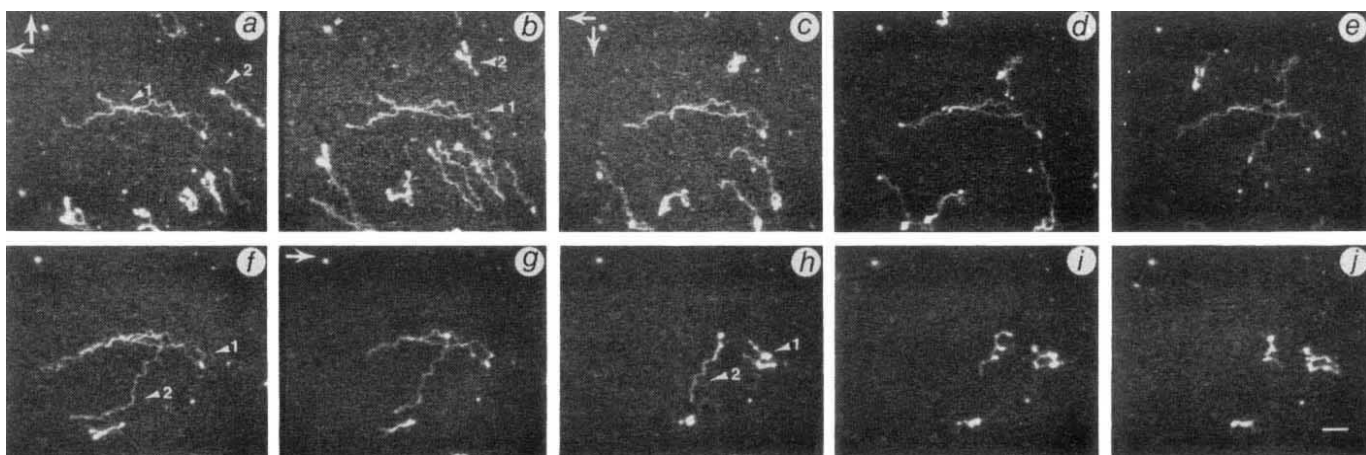


FIG. 2 Bacteriophage G DNA using POE conditions analogous to those for pulse field electrophoresis with a 90° field angle (3, 3–84 s at 3.5 V cm^{-1} ; see Methods), followed by field reversal (continuous field; 3.5 V cm^{-1}). a–f, Images obtained at 570 s (a), 600 s (b), 612 s (105 after field switch) (c), 624 s (d), 672 s (e), and 836 s (f) after POE-field initiation. g–j, Images obtained at 2 s (g), 6 s (h), 12 s (i) and 18 s (j) after field reversal. a, The trapped molecule (1) joined by other coils moving into the field from the bottom; b, the newly arrived coils display conformations including hooks and condensed coils. c, After the field switch (arrows), hooks resolve into single strands terminated with a dense region. The newly entangled coil (2) undergoes considerable conformational change from a thick rod (a), to a

stubby hook (b), to a round, dense mass (c). The two trapped coils (d–f) show the ‘staircase’ effect characteristic of POE. g–j, Pulsing ends and a reversing field is applied, which rapidly unhangs, disentangles and collapses both molecules. Scale bar, $10 \mu\text{m}$.

METHODS. Microscopy and sample preparation methods are described in Fig. 1. POE utilized a series of short, alternating perpendicular pulses. After a period of time, the polarity of one of the fields was switched. 3, 3–84 s describes a cycle, alternating between a 3 s pulse east–west and a 3 s pulse south–north with an 84 s duration, followed by another 84 s cycle alternating between a 3 s pulse east–west and a 3 s pulse north–south and so on.

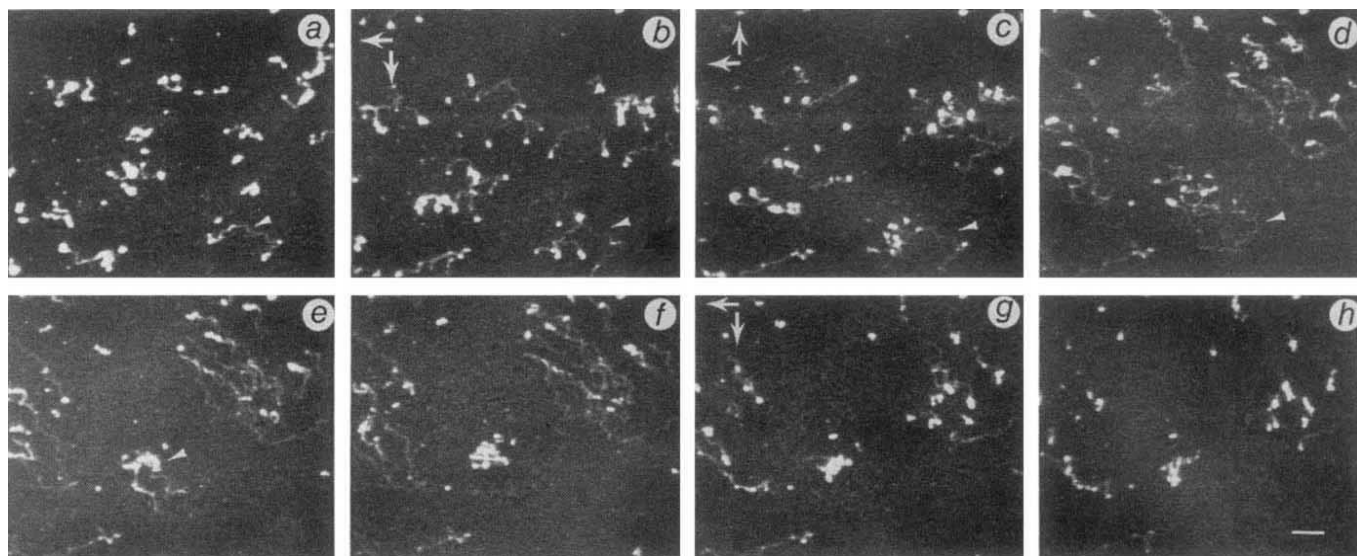


FIG. 3 G bacteriophage DNA electrophoresis with POE conditions emulating an obtuse field angle; 6, 10–80 s, at 3 V cm^{-1} . *a–h*, Images obtained 0 s (*a*), 18 s (*b*), 85 s (3 s after field switch) (*c*), 120 s (*d*), 138 s (*e*), 158 s (*f*), 165 s (5 s after field switch) (*g*) and 171 s (*h*) after POE-field initiation. Changes in field orientation are shown by arrows (*b, c, g*). Molecules begin from a

relaxed conformation (*a*) and elongate to a 'staircase' conformation characteristic of POE as the pulsing proceeds (*b–h*). Inversion of the hooks also occurs (*c–e*; arrowhead). Scale bar, $10 \mu\text{m}$. Sample preparation and experimental conditions were as described in Figs 1 and 2.

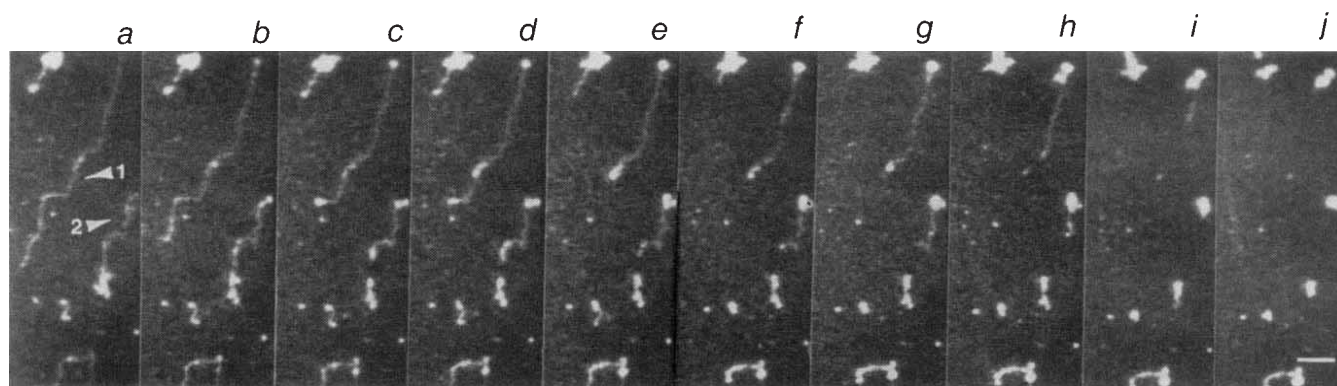


FIG. 4 Relaxation of G bacteriophage DNA molecules in the absence of an electric field. Molecules were initially electrophoresed for 600 s (POE conditions; 3, 5–80 s, 3 V cm^{-1}). Images, obtained at 12 s intervals, show the relaxation of several molecules over a 111-s time span. *a*, 3 s after turning off the applied field. The coils (arrowheads) relax through the same 'staircase'

path that was induced by the applied electrical pulses (as determined by the limits of microscopic resolution). *c*, Note that either one molecule (molecule 2) has fragmented or two molecules have separated. *j*, All coils have relaxed to a round, unelongated conformation. Scale bar, $10 \mu\text{m}$. Sample preparation and experimental conditions were as described in Figs 1 and 2.

augment coil relaxation in that direction (in hooks, this is towards the apex). Additionally, the coil ends may not be equivalent, which may also bias motion in both perpendicular and obtuse field situations.

Despite the low sample concentrations used in the electrophoresis experiments, DNA–DNA interactions were also observed. Figure 2, for example, shows a temporarily immobilized DNA molecule (molecule 1) trapping another DNA molecule. Reversal of the electric field (180° field shift) released both molecules and caused the rapid collapse and eventual

reformation of the hooks. It is probable that similar events occur in reverse-field electrophoresis and this is, indeed, the conclusion of several computer simulations^{3,4,6} and spectroscopic studies¹³.

These results show that static-field, pulse-field and pulse-oriented electrophoresis give rise to similar cycles of DNA conformational changes that include hook formation, resolution, collapse and re-formation. Furthermore, it seems that the field angle has a pronounced effect on DNA coil dynamics with perpendicular field angles preventing the rapid collapse of structure after field switching. □

Received 21 November 1988; accepted 21 February 1989.

1. Yanagida, M. *et al.* in *Applications of Fluorescence in the Biomedical Sciences* (eds Taylor, D. L., Waggoner, A. S., Murphy, R. F., Lanni, F. & Birge, R. R.) 321–345 (Alan R. Liss, New York, 1986).
2. Matsumoto, S., Morikawa, K. & Yanagida, M. *J. molec. Biol.*, **152**, 501–516 (1981).
3. Deutsch, J. M. *Phys. Rev. Lett.* **59**, 1255–1258 (1987).
4. Deutsch, J. M. *Science* **240**, 922–924 (1988).
5. Violy, J. L. *Phys. Rev. Lett.* **60**, 855–858 (1988).
6. Zimm, B. H. *Phys. Rev. Lett.* **61**, 2965–2968 (1988).
7. Schwartz, D. C. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **47**, 189–195 (1983).
8. Schwartz, D. C. & Cantor, C. R. *Cell* **37**, 67–75 (1984).

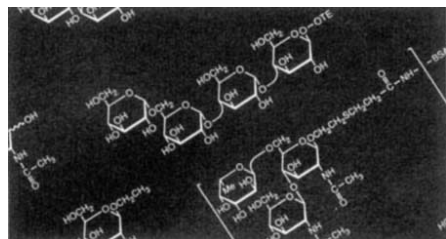
9. Schwartz, D. C. thesis, Columbia Univ. (1985).
10. Clark, S. M., Lai, E., Birren, B. W. & Hood, L. *Science* **241**, 1203 (1988).
11. Chou, G., Vollrath, D. & Davis, R. *Science* **235**, 1582–1585 (1986).
12. Schwartz, D. C., Koval, M. & Hsu, M. manuscript in preparation.
13. Holzwarth G., McKee, C. B., Steiger, S. & Crater, G., *Nucleic Acids Res.* **15**, 1031–1044 (1987).
14. Fangman, W. L., *Nucleic Acids Res.* **5**, 653–665 (1978).

ACKNOWLEDGEMENTS. We thank Dr Richard Pagano for his support, Dr Bruno Zimm for discussions, and Cindi Smith, Mei Hsu, Tony Ting and Connie Jewell for assistance. This work was supported by the NIH and the Lucille P. Markey Charitable Trust. D.C.S. is a Lucille P. Markey Scholar.

Springtime launches for the lab

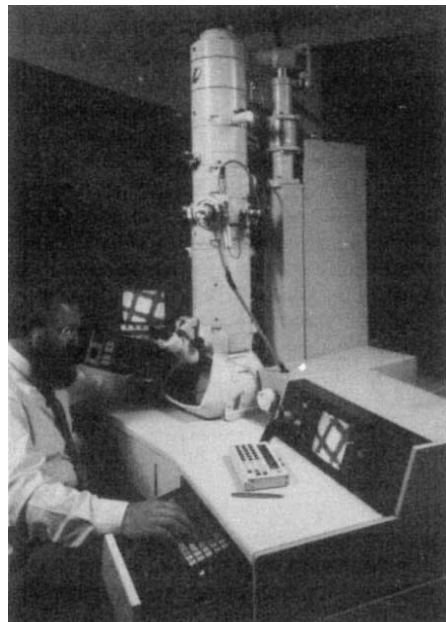
The spring of the year brings a flurry of product introductions: this season's best includes a phenol-free DNA extraction kit, an LC/MS that can handle haemoglobin, and a benchtop robot that does the drudge jobs without complaint.

SIGMA Chemical Company recently announced a special line of highly purified synthetic oligosaccharides and derivative



Bioactive carbohydrates from Sigma Chemical. fragments of biologically active glycoconjugates (*Reader Service No. 100*). The complex **bioactive carbohydrates** have applications in diverse areas of medical and biological research, including substrate specificity studies of glycosidases and glycotransferases, and studies of drug targeting, cell proliferation and oncogenesis. They may also be used as model compounds for the study of complex carbohydrates by NMR spectroscopy, and can be incorporated into liposomes.

Jeol's new model JEM 1200 EXII **electron microscope** was developed specifically to support fundamental biotechnology research and environmental studies involving particle microanalysis (*Reader Service No. 101*). The microscope has a three-stage, six-lens image-forming system, which Jeol says provides high resolution, a wide visual field, low image



Jeol's new transmission electron microscope has a wide magnification range.

distortion and high-contrast image quality at low magnifications. The TEM has a magnification range of 50–1,000,000 ×, and a focus zoom which allows magnification changes without focus changes. Samples may also be measured quantitatively with Jeol's microscope: lengths, angles, and areas of the overall sample can be recorded, and lattice spacing and angles can be determined using diffraction patterns, down to a resolution of 0.14 nm.

Oncor says its new non-organic **DNA extraction kit** produces high-purity, high-yield DNA in four hours, without the use

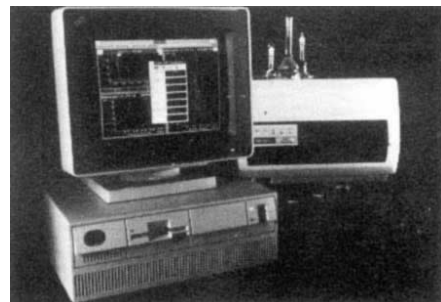


No fussing with phenol is necessary with Oncor's non-organic DNA extraction kit.

of hazardous organic chemicals (*Reader Service No. 102*). The kit's reagents are qualified on human samples in Oncor's molecular pathology laboratory. The company says the kit provides DNA whose purity compares with phenol extraction: Oncor says the absorbance ratio (A_{260}/A_{280}) of the purified DNA falls between 1.75 and 1.90. The kit eliminates the need for dialysis or extraction steps, cutting DNA purification time down from three days to roughly four hours, Oncor says. The proprietary reagents used in the \$150 (US) DNA extraction kit are non-caustic and non-carcinogenic, and can be poured safely down laboratory drains.

The computing edge

PolyView is the name of a new software system from Varian which aims to make **diode array spectral processing** for liquid chromatography more productive (*Reader Service No. 103*). The PolyView software is designed to work with the Polychrom 9065 diode array detector, as a part of a complete \$23,000 (US) system built around the PC-based LC Star chromato-



PolyView chromatogram analysis software.

graphy workstation. With the Microsoft Windows environment of PolyView, chromatographers can take data from an information-rich detector, and present it in a variety of diverse formats simultaneously on one computer screen. The software has a bidirectional library function: users can search the library for a chromatogram that matches peaks from one obtained experimentally, or search a given chromatogram for similarities to a library standard. Reports are printed automatically at the end of each run. PolyView also calculates Varian's Purity Parameter — the average wavelength weighted by the square of the absorbance — to help chromatographers locate impurities.

Mathor 3, the **technical word processing software** from Novedit, displays scientific formulae and mathematical equations on the computer screen as they will appear in printed form (*Reader Service No. 104*). The software automatically draws and sizes all mathematical symbols, such as square root signs and integrals, to accommodate their contents. Formulas can be inserted on the same line as text, and frequently used mathematical expressions can be stored in Mathor 3's memory to be inserted at the touch of a key. Mathor 3 has five character sets, including the full Greek alphabet, operators and logical symbols, and common physics and mathematics characters. Text may be imported to Mathor 3 from Word and WordPerfect word-processing programs. The FF3,000 (France) Mathor 3 software runs on IBM PC and PS/2 computers equipped with a graphics adaptor card.

The LabSolutions program from The Center for Science Support, Inc. automatically performs the necessary calculations for preparing multi-component solutions and buffers (*Reader Service No. 105*). The \$99 (US) **solution calculation software** uses standard units of weight,

volume, and concentration, and calculates molecular weights from atomic formulas or chemical names. Solution recipes can be stored on disk for retrieval, and printed out for documentation purposes. Specialized routines for mixing and diluting solutions, and for automatically generating linear and geometric dilution series are also included. Users may add their own entries to the built-in databases of chemicals, buffers, acids and bases. The LabSolutions user manual includes chapters on choosing the best buffer, deriving titration equations, and adjusting the pH. LabSolutions is available on 3.5-in. and 5.25-in. diskettes for IBM PCs; a Macintosh version will be released soon.

Popular culture

Laboratory Impex Ltd has a **Mycoplasma detection kit** which it says sniffs out the presence of as little as 10^5 organisms contaminating a tissue culture in just three hours (*Reader Service No. 106*). The company's Gen-Probe Mycoplasma TC detection system employs the principle of nucleic acid hybridization: the kit contains a ^3H -labelled DNA probe homologous to Mycoplasma and Acholeplasma ribosomal RNA. Laboratory Impex says the probe detects all species which commonly infect tissue cultures. Incubating the hybridization reaction for 15–20 hours increases the sensitivity of the test three to five times, according to the company.

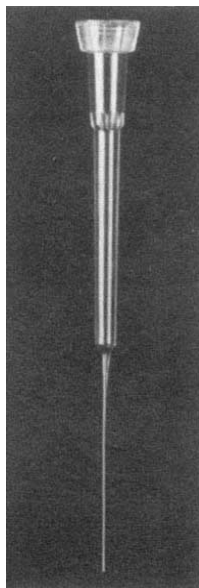
Baxter Healthcare Corporation has a flexible, 25-litre **recovery container** designed to replace carboys and stainless steel tanks in large-scale cell culture (*Reader Service No. 107*). The Lifecell container is composed of several layers of non-toxic, gas-barrier plastic which Baxter says offers stability equivalent to that of glass. The containers require minimal storage space, and can be stacked in tote pans when filled. Baxter says the container is lined with a layer of polyolefin which is non-reactive with biologicals. Two separate tubes with Luer connection ports are attached to the container to facilitate filling and emptying procedures.

Gel gems

Bio-Rad's roundup of electrophoresis products includes **pipette tips for gel loading** and a **reagent and sample preparation**



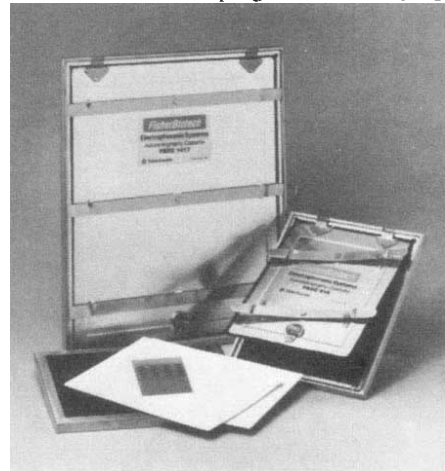
Bio-Rad's kit for reading samples and reagents for electrophoresis.



Bio-Rad's Gel-Loader.

kit (*Reader Service No. 108*). Bio-Rad's Gel Loader disposable capillary pipette tips have a non-wetting surface which delivers samples of between 0.5 and 10 μl . The capillary of each tip is less than 0.35 mm o.d., making them useful for loading 0.25-mm sequencing gels. The 15-mm capillary reaches to the bottom of each well in a gel, eliminating cross-contamination between lanes. Before wielding the pipette, Bio-Rad recommends cleaning up both samples and reagents with its kit which contains all of the materials needed to remove Triton X-100 detergent and SDS from samples, to concentrate 0.5–1.5-ml samples, and to deionize impure urea, formamide, glyoxal or acrylamide. Bio-Rad says use of the kit can prevent band broadening and reduce background levels.

The FisherBiotech division of Fisher Scientific has a new line of **autoradiography cassettes** for developing and intensifying

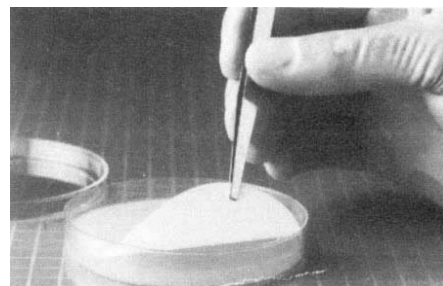


Protective cassettes for autorads from Fisher.

autoradiographs from electrophoretic gels (*Reader Service No. 109*). The cassettes come in sizes of 8 × 10 inches, 14 × 17 inches and 14 × 36 inches. All have stainless-steel frames and felt liners to prevent light leakage and ensure optimal contact between film and screen. The doors of the cassettes have integral locking bars, and Fisher says the cassettes are virtually warp-proof. The 8 × 10-inch cassettes cost \$88 (US); \$96 (US) with screens.

Probing analyses

Micron Separations, Inc. is giving away an application kit for reprobing its NitroPlus 2000 supported nitrocellulose membranes



Get a free sample of Micron Separation's supported membranes.

(*Reader Service No. 110*). The **free membrane reprobing kit** contains a sample of MSI's NitroPlus 2000 membrane, two newly developed techniques for reprobing the membrane, and detailed procedures for stripping and reprobing Northern and Southern blots up to six times. Micron Separations says 80–90 per cent of the signal from the target DNA or RNA is retained using the reprobing protocols, with 90–100 per cent of the probe being removed after each reprobing step.

BRL has a new system for simplifying and standardizing the procedure of **labeling DNA probes** with biotin by nick translation (*Reader Service No. 111*). BRL's BioNick labelling system generates small probes which range in size from roughly 50 to 500 base pairs. Because they are small enough to penetrate tissue easily, BRL says the probes are ideal for *in situ* hybridization. The BioNick system employs a new biotinylated nucleotide, biotin-14-dATP, which has a long, 14-atom linker arm that BRL says leaves enough room for streptavidin to bind for the identification of probe-target hybrids. The BioNick kit includes sufficient reagents for labelling 50 μg of DNA: a 10 × dNTP mixture that contains biotin-14-dATP in optimal concentration, a 10 × enzyme solution, stop buffer, autoclaved water, a positive control DNA to monitor performance, and detailed instructions.

Handy helpers

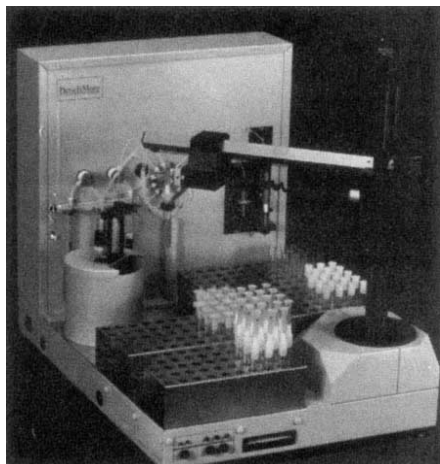
BioTherm Corporation has a \$2,990 (US) **thermal cycler** for performing enzymatic gene amplification and other variable-temperature protocols (*Reader Service No. 112*). BioTherm's BioOven has an open design which allows it to be used with microtitre plates, microcentrifuge tubes, and a variety of other sample containers.



Gene amplification is just one of many applications for the air-heated oven from BioTherm.

The BioOven uses air heat, eliminating water spills and the need for oil layering. BioTherm says the oven heats from ambient temperature to 100 °C in roughly one minute, with a temperature uniformity of 1 °C. The oven's built-in micro-processor allows six independent heating and holding cycles within a given routine; up to eight separate programs can be stored and recalled for later use.

The BenchMate **benchtop robot** from Zymark was designed to free researchers from performing the routine sample preparation tasks required in most laboratories (*Reader Service No. 113*). The BenchMate can be configured to dispense liquids, perform membrane filtrations and solid-phase extractions, and inject samples into an HPLC for analysis. Zymark's \$11,000–18,000 (US) robot takes up just 21.5 × 29.5 inches of bench space, and holds up to 200 standard-sized test tubes. Precision syringes with Zymark's 12-port valving technology dispense up to 10 ml of liquid; with the gravimetric confirmation option, an audit trail can be created to confirm all liquid deliveries by weight. The BenchMate is



Zymark's answer to monotonous lab procedures: BenchMate.

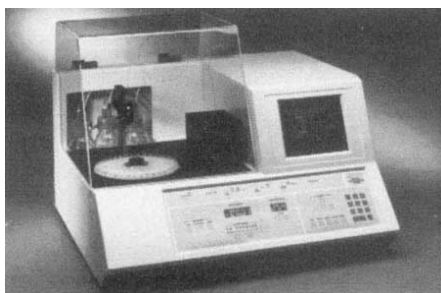
controlled by a 3.5-in diskette which contains a menu-driven operating system for building method files, and can be programmed by direct connection to an IBM PC.

Analytical technology

Last month Finnigan MAT introduced a **benchtop GC/MS** system which combines complete spectrum separation with detection at the picogram level — the Ion Trap System 40 (*Reader Service No. 114*). The ITS 40 is targeted for the environmental and forensic/clinical markets, where low-level trace identification is important. The system consists of the Varian model 3400 gas chromatograph mounted on a single base plate with the Finnigan ion trap quadrupole mass spectrometer, and controlled by an integrated Compaq Deskpro computer running Finnigan MAT's proprietary software. Finnigan MAT says the

\$85,000 (US) ITS 40 system supports a full-spectrum acquisition rate of up to 10 spectra per second and a scan rate greater than 5,600 μs^{-1} .

Dionex has the latest entrant onto the market of the hottest analytical technique of the year: capillary electrophoresis (*Reader Service No. 115*). The Dionex CES I **capillary electrophoresis** system has detectors for both UV/visible absorbance and fluorescence which are linked to the instrument by fibre optics, bringing the



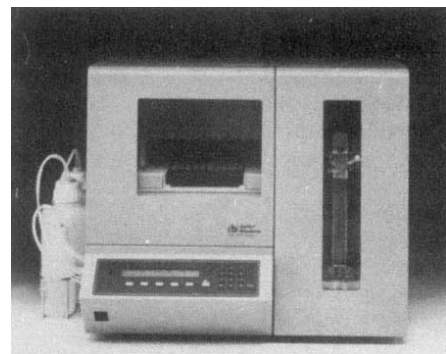
The Dionex system for capillary electrophoresis.

detector closer to the column. The CES I's 40-place autosampler is capable of performing the three most popular injection methods: electromigration, gravity and pneumatic injection. An automatic rinse and refill buffer system allows buffers to be changed during runs, and ensures reproducible electrophoresis by using fresh buffers for each run, says Dionex. The spring-loaded design of the cell means that capillaries can be changed quickly. The \$37,500 (US) system is controlled by menu-driven software and a video display. Results are generated in a chromatography-like format, so the system can interface with any recorder or integrator.

Sciex has developed an LC/MS system especially for the needs of the biopharmaceutical industry (*Reader Service No. 116*). Sciex's API III joins HPLC together with tandem mass spectrometry, through an atmospheric pressure ionization interface that Sciex says outperforms fast-atom bombardment and thermospray ionization methods to allow femtomolar detection. The IonSpray interface desorbs ionic compounds in the solution eluting from the HPLC into the ionization source using field-assisted ion evaporation. The molecular ions are then transferred to the mass analyser through a nitrogen curtain gas. The \$395,000 (US) system can be used to glean sequence information from peptides, proteins and oligonucleotides with molecular weights of over 100,000: Sciex says the API III has been used successfully to analyse haemoglobin.

Setting it straight

Contrary to an earlier description (see *Nature* 338, 94; 1989), the model 230A HPEC system from Applied Biosystems is not a capillary electrophoresis instrument.



Automated electrophoresis, not CZE.

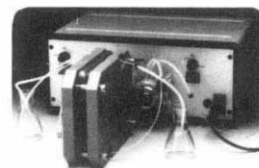
HPEC stands for high-performance electrophoresis chromatograph: the model 230A HPEC is an automated micro-preparative gel electrophoresis system which isolates and quantifies proteins and DNA on a tube gel column using standard electrophoresis protocols (*Reader Service No. 117*). After detection, eluted compounds pass into an integral fraction collector. Applied Biosystems's newly launched analytical capillary electrophoresis system is model number 270A. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

ADVERTISEMENTS

LIPOSOMES

REPRODUCIBLE — HOMOGENEOUS —
EXTREMELY RAPID



Our new LIPOSOMAT guarantees rapid (5ml within 60 minutes) preparation of uniformly sized unilamellar liposomes. Liposome size is selectable between 25 and ca. 600 nm diameter. Sample volume is normally 5–10ml or up to 200ml if used in combination with an additional instrument. Lipid concentration can be up to 300 mg/ml.

DIABORM

POB 126, D-8000 Munich 65, FRG

Reader Service No.45

Medwire Div.

stocks a wide variety of

TEFLON COATED*

Platinum alloys, stainless steel, silver, multistrand and single strand available for prompt shipment. Write for descriptive brochure and price lists.



Medwire Div.

Affiliate of Sigmund Cohn Corp.

123 So. Columbus Ave.
Mt. Vernon, N.Y. 10553
914-664-5300

*Reg. T.M. of E.I. DuPont

Reader Service No.46

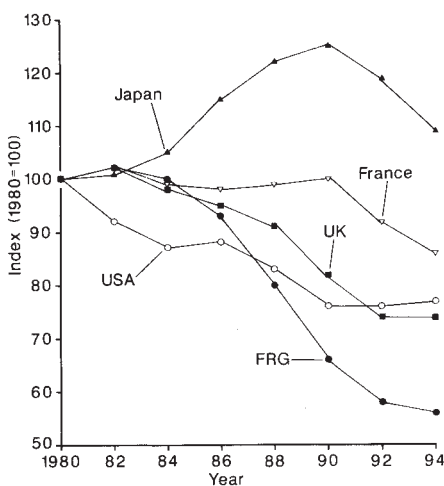
The impact of Europe's 1992

Richard Pearson

Business links between Europe and the United Kingdom are increasing and barriers to mobility of labour are coming down. There are new opportunities for both employers and individuals.

As the date for closer European integration draws near, increasing attention is being paid to the possible effects it may have on UK and other European labour markets. International companies are developing management teams which can work across international boundaries. Others are moving into overseas markets for the first time and having to build up a knowledge of local employment conditions and styles of operating. Yet other companies are expanding their recruitment market across national borders.

Individuals are also increasingly moving across borders as horizons of opportunity are broadened from the traditional North American and Commonwealth job markets (*Nature* 319, 84; 1986). For example, we have recently seen a significant inflow of veterinarians into the United Kingdom from Italy and other parts of Europe impacting on the job opportunities of UK graduates. Even those organizations operating solely within the United Kingdom, for example the universities, polytechnics and research institutes, are being affected as European recruiters target the UK labour market. Already Siemens,



Index of populations of 16-18-year-olds, taking 1980 as the baseline (index 100). Source: *International Science and Technology Update* (NSF, Washington, 1986).

Thompson and Philips are among the regular recruiters in the United Kingdom.

In the United Kingdom skill shortages are going to get worse, because the downturn in the number of young people entering the labour market over the next five years will be even more disruptive if the planned economic growth continues. So are things easier in Europe? The first point is that the demographic downturn is

a near-universal phenomenon, hitting for example France, Italy, the Netherlands and West Germany alike; in the latter case the downturn is as high as 45 per cent over a decade (see figure), with only Ireland having an expanding youth labour force. We see also a convergence across labour markets with common growth occupations at high skill levels and in the services sector, and declining opportunities for the unskilled and manual workers.

Because of differences in structure and content, comparisons of the numbers graduating have to be made with care. For example, while fewer students in the United Kingdom are enrolled in higher education pro rata to population size, the numbers graduating compare more favourably because of the lower drop-out rates, and although the United Kingdom does not compare so well in terms of engineering it does rather better in the sciences. In terms of the balance between supply and demand we see a segmented market in many countries with high levels of graduate unemployment in the Netherlands, Spain, West Germany, Italy and Ireland coexisting with shortages of engineers, information technology specialists, and business studies graduates.

So how easy will it be to recruit in Europe? The free mobility of labour in the Community means work permits are no longer a problem, although Portugal and Spain will not be included until 1993. Another change about to make itself felt is the mutual recognition of qualifications. Mutual recognition for pharmacists has recently been achieved, but only after 16 years of negotiations, and that for architects after 17 years. A more specific problem will be knowing where to go to recruit and being able to make judgements about the European graduates. For example, while in the United Kingdom the careers services actively help recruiters by 'selling' their graduates, in many parts of Europe such services are rare and in some cases direct recruitment is banned on campuses. In these cases use has to be made of more general national employment services. Recruitment is more usually carried out through personal links with academics, student work placements and direct advertising in the national newspapers and professional journals. Speculative applications from students are also much more common. In Italy, family and personal contacts are also important, factors reinforced by the much greater tendency of students to live at home and go to local

universities, a practice which is common in continental Europe. Living away from home seems to be peculiar to Britain and to students attending national, prestigious institutions in Europe. A further complication for the outside recruiter is the problem of judging the relative merits and qualities of institutions, for example, comparing Louvain with Berlin and Barcelona.

New employment strategies will also be required for the European graduate, who will typically be 4-5 years older than the UK equivalent and will have come from a broader-based, although still specialist, degree course. Different selection tests may be needed, certainly less reliance can be placed on extracurricular activities, which are far less common, and on academic references, which are not normally available on the continent. As most European graduates will be aged 26-28, because of their longer degree courses and national service, they are likely to be more mature and capable than the 21-year-old UK graduate. Initial jobs are therefore likely to be at a higher level in an organization. Induction and initial training needs are therefore also likely to be different, as will the starting salary. In part these differences help explain the discrepancies that are quoted between UK and European graduate starting salaries.

Many challenges face the British recruiter looking to Europe as a new recruitment source. At the same time European recruiters are starting to target the United Kingdom so we will not have exclusive use of the home market. Competition is likely to be greatest in the case of the extended engineering degrees, some of the applied sciences and business courses, particularly where students have a foreign language. Few European recruiters are interested in the generalist graduate. A dilemma for our careers services will be whether, in the interests of serving their prime customers, the students, they encourage and welcome European recruiters on to the campuses.

This increasing internationalization of business and labour markets will increase mobility both into and out of the United Kingdom. European recruitment is not, however, going to be an easy solution to UK shortages. The challenge for the 1990s will be to ensure that European integration offers new opportunities for individuals and employers and does not just mean a new brain drain. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton. BN1 9RF, UK.